SOL2015

The 12th Solanaceae Conference

October 25 -29, 2015
ENSEIRB Building, Talence

Bordeaux, France

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The 12th Solanaceae Conference

SOL2015

Book of Abstracts

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- Dr Mathilde CAUSSE (INRA Genetics and Improvement of Fruit and Vegetable, France)
- Pr Mondher BOUZAYEN (INRA/INP ENSA Toulouse, France)

Conference Chairs and Speakers (alphabetic order):

- Pr Asaph AHARONI (Weizmann Institute of Science, Israël)
- Pr Tohru ARIIZUMI (Tsukuba University, Japan)
- Dr Christian BACHEM (Wageningen University, Netherlands)
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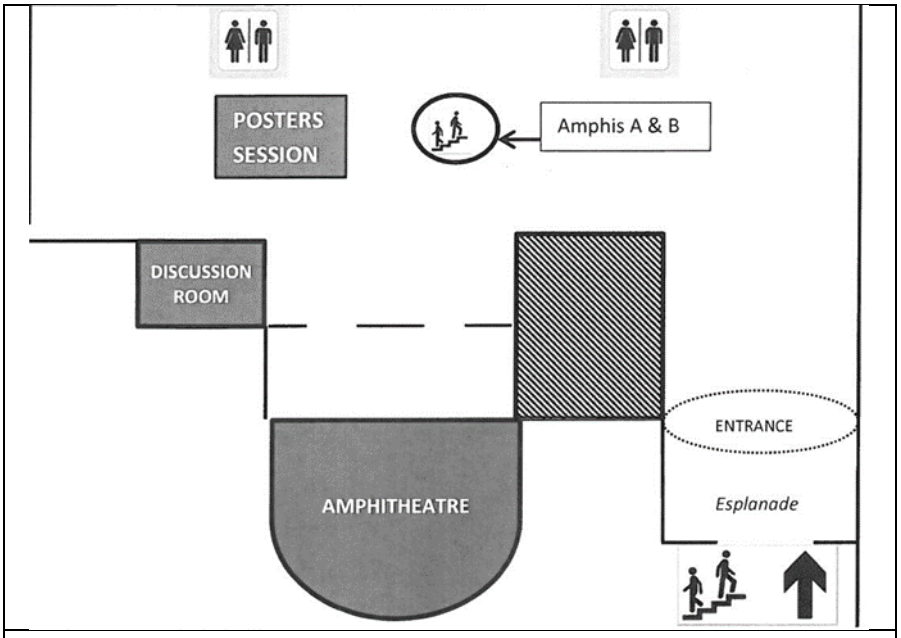
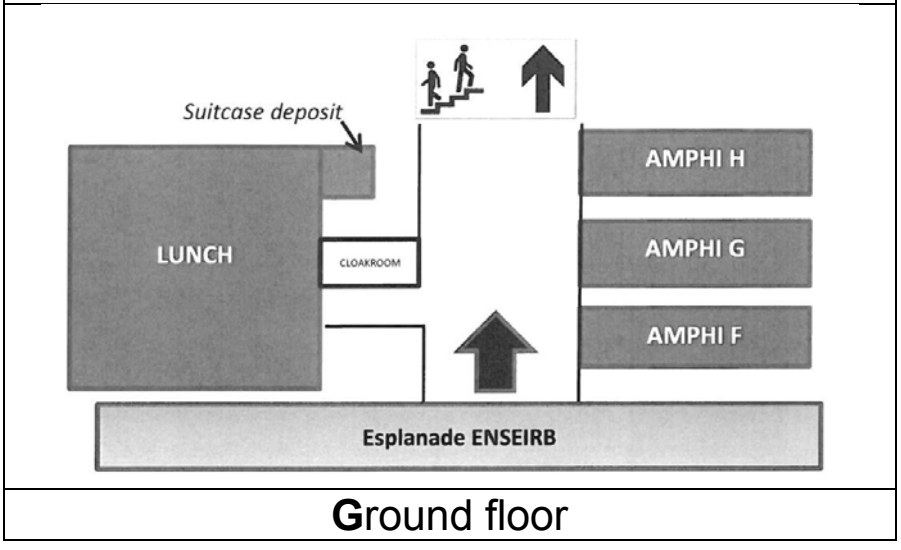
- Pr Chuanyou LI (Chinese Academy of Sciences, China)
- Pr Zachary LIPPMAN (Cold Spring Harbor Laboratory – CSH, USA)
- Pr Lukas MUELLER (Boyce Thompson Institute, Cornell, USA)
- Dr Zoran NIKOLOSKI (Max Planck Institute of Molecular Plant Physiology, Golm, Germany)
- Dr Ilan PARAN (Agricultural Research Organization, Israël)
- Dr Francisco PEREZ-ALFOCEA (CEBAS-CSIC Murcia, Spain)
- Pr Jocelyn ROSE (Cornell University, USA)
- Dr Thomas STÄDLER (Swiss Federal institute of Technology –ETH, Switzerland)
- Pr Miltos TSIANTIS (Max Planck Institute for Plant Breeding Research, Köln, Germany)

IPB ENSEIRB MATMECA BORDEAUX

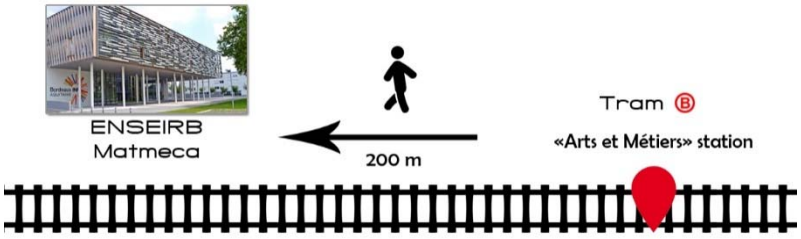
1, avenue du Dr A. Schweitzer
33402 Talence



First floor



Ground floor

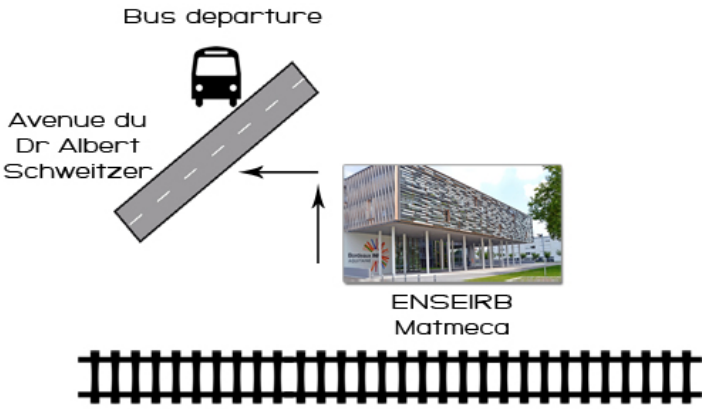


Tue, Oct 27th 14:00-18:00

Excursion in **Saint Emilion** the medieval town of Saint Emilion, in the heart of the Bordeaux wine world

Only for people with the logo  on their badge. A ticket will be given if you choose to do the St Emilion visit and this ticket must be given to the guide on the bus.

The place of departure will be near the building of the ENSEIRB, streetside, at avenue du Docteur Schweitzer.



Wed, Oct 28th 20:00

The **Gala dinner** will be organized in one of the most prestigious place of Bordeaux, the **Palais de la Bourse**, located in the heart of the city by the river, benefiting from an ideal situation, easily reachable with all types of transports.

The Palais de la Bourse is surrounded by historical buildings, which reflect the cultural heritage of the city.

Pictures Credits : Palais de la Bourse.

Address : 19 Place de la Bourse, Bordeaux

PROGRAM AT A GLANCE

Sun, Oct 25		Mon, Oct 26		Tue, Oct 27		Wed, Oct 28							Thu, Oct 29		
		08:00	Registration												
		08:45-09:00	Opening Welcome	08:45-09:30	*Keynote lecture* *J. Giovannoni*	08:45-09:30	*Keynote lecture* *Z. Nikoloski*						08:45-09:30	*Keynote lecture* *A. Levy*	
		09:00-09:45	*Keynote lecture* *M. Tsiantis*												
		09:45-10:15	**I-Plant Growth & Dev.** *Z. Lippman*	09:30-10:00	**V-Flower, Fruit & Tuber Biology** *C. Chevalier*	09:30-10:45	**VI-*Metabolism* and Quality** *A. Aharoni*						09:30-10:30	**VIII-New tools for gene discovery & biotechnology** *A. Granell*	
		Coffee Break		*Coffee Break & Poster session*		*Coffee Break & Poster session*							*Coffee Break & Poster session*		
		10:45-12:00	**I-Plant Growth & Dev.**	10:30-12:00	V-Flower, Fruit & Tuber Biology	11:15-12:45	**VII-Systems Biology and Modelling** *J. Rose*						11:00-12:00	**IX-Biotic stress I** *C. Li*	
		Lunch		*Lunch and Poster session*		*Lunch & Poster session*							*Lunch*		
		14:00-15:45	**II-Biodiversity** *T. Städler*	14:00-18:00	Excursion in Saint Emilion	**PARALLEL SESSIONS(*)**							**SESSIONS X and XI**		
						Amphi	0	F	G	H	A	B	Amphi	0	F
						14:15-15:45	**TO** I	**PO** I	**CO** I	**PE** I	**SM** I	**PSQ**	14:00-15:30	**X-Biotic stress II** *Y. Bai*	**XI-Abiotic stress** *F. Perez-Alfocea*
16:00-20:00	Regis-tration Welcome reception	*Coffee Break*				*Coffee Break*							15:30	Closing of SOL2015 and presentation of SOL2016	
		16:15-17:30	**III- Molecular Breeding** *G. Bryan*			16:15-17:30	**TO** II	**PO** II	**CO** II	**PE** II	**SM** II	**TB**			
		17:30-18:45	**IV-Bioinformatics & SGN Workshop** *L. Mueller*												
		18:30-20:30	*Poster session & wine tasting*			*20:00*	*Gala Dinner*								

(*)PARALLEL SESSIONS : **TO**: Tomato (M. Bouzayen/T. Ariizumi) **PO**: Potato (C. Bachem) **CO**: coffee (A. de Kochko / D. Crouzillat) **PE**: Pepper/Eggplant (I. Paran/ D. Choi)
SM: Specialized Metabolism (R. Last) **PSQ**: Photosynthesis and Fruit Quality (J. Harbinson) **TB**: Tobacco (N. Ivanov)

Monday, October 26th

08:45-09:00	Opening Welcome	
09:00-09:45	**Miltos Tsiantis** - *Keynote lecture* Towards understanding development and diversity of leaf shape	KL-1
Session I : PLANT GROWTH AND DEVELOPMENT Chair : **Zachary Lippman** *(Cold Spring Harbor Laboratory (CSH), US)*		
09:45-10:00	**Bettina Hause** Stamen development in tomato – jasmonates control ethylene action in anther dehiscence and pollen release	OC-1
10:00-10:15	**Niels A. Müller** The circadian clock of cultivated tomato has been slowed down during domestication	OC-2
10:15-10:45	*Coffee Break*	
10:45-11h00	**Michael Nicolas** How to make it branched: a recently evolved alternative splice site in BRANCHED1a controls plant architecture in potato	OC-3
11:00-11:15	**Klaus Theres** Boundaries shape tomato shoot architecture and initiate new meristems	OC-4
11:15-11h30	**Kieron Edwards** A time to flower: Insights into the photoperiodic regulation of flowering in Tobacco	OC-5
11:30-12:00	**Zachary Lippman** - *Invited lecture* Returning to domestication to revitalize crop improvement: engineering the florigen system	SP-1
12:00-14:00	*Lunch*	
Session II : BIODIVERSITY Chair : **Thomas Staedler** *(Swiss Federal Institute of Technology (ETH), Switzerland)*		
14:00-14:30	**Thomas Staedler -** *Invited lecture* Using molecular diversity in wild tomatoes for inferences on evolution and speciation	SP-2
14:30-14:45	**Jose Blanca** Genomic variation in tomato, from wild to cultivated	OC-6
14:45-15:00	**Xavier Aubriot** Working out the history and the biogeography of Old World "spiny solanums" (*Solanum* subg. *Leptostemonum*): the key contribution of the overlooked Tropical Asian species	OC-7
15:00-15:15	**Rafael Costa-Silva** Taxonomic application of the leaf anatomy in species of *Solanum* clades *Gardneri* and *Thomasiifolium*	OC-8
15:15-15:30	**Aureliano Bombarely** Evolutionary analysis of Petunia genomes	OC-9
15:30-15:45	**Michael Hardigan** Genome reduction suggests large dispensable genome and adaptive role for copy number variation in asexually propagated *Solanum tuberosum*	OC-10
15:45-16:15	*Coffee Break*	

Session III : MOLECULAR BREEDING Chair : **Glenn Bryan** *(The James Hutton Institute, Dundee, UK)*		
16:15-16:45	**Glenn Bryan** – *Invited lecture* Recent advances in development of tools for potato genetics and genomics	SP-4
16:45-17:00	**Christopher Sauvage** Assessment of factors affecting genomic selection in populations of tomato	OC-11
17:00-17:15	**Pedro Almeida** Genomics-assisted selection of *Solanum chilense* introgression lines for enhancing drought resistance in tomatoes	OC-12
17:15-17:30	**Morgane Roth** Understanding hybrid seed failure in wild tomatoes: phenotypic and transcriptomic signatures	OC-13
Session IV : BIOINFORMATICS and SGN Workshop Chair : **Lukas Mueller** *((Boyce Thompson Institute, USA)*		
17:30-17:45	**Richard Finkers** Genebanks and Genomics: how to interconnect data from both communities?	OC-14
17:45-18:00	**Kenta Shirasawa** In silico and empirical optimizations of ddRAD-Seq for tomato genomics, genetics, and breeding	OC-15
18:00-18:45	**Lukas Mueller – SGN Workshop**	
18:30-20:30	*Poster session and wine tasting*	

Tuesday, October 27th

08:45-09:30	**Jim Giovannoni** – *Keynote lecture* Genetic factors influencing tomato fruit ripening	KL-2
Session V : FLOWER, FRUIT AND TUBER BIOLOGY Chair : **Christian Chevalier** *(INRA, UMR BFP, Bordeaux, France)*		
09:30-10:00	**Christian Chevalier** - *Invited lecture* DNA-dependent fruit growth in tomato: endoreduplication and the karyoplasmic ratio theory	SP-5
10:00-10:30	*Coffee Break and Poster session*	
10:30-10:45	**Sebastian Soyk** Engineering the florigen pathway in tomato	OC-16
10:45-11h00	**Eder M. Silva** Over-expression of SlyMIR159 alters flower development and promotes parthenocarpic fruit formation in tomato	OC-17
11:00-11:15	**Jean Pierre Renaudin** The coordinated pattern of cell division and cell expansion during fruit set and fruit growth in tomato pericarp	OC-18
11:15-11h30	**Liu Re** Active DNA demethylation controls tomato fruit ripening	OC-19
11:30-11:45	**Yonatan Elkind** "Rocky" – A novel delayed ripening pepper mutant	OC-20
11:45-12:00	**Graham B. Seymour** Cloning of a gene underlying a major texture QTL in tomato	OC-21
12:00-14:00	*Lunch and Poster session*	
14:00-18:00	Excursion in Saint-Emilion	

Wednesday, October 28th

08:45-09:30	**Zoran Nikoloski** - *Keynote lecture*. Integration of high-throughput data in models of plant metabolism	KL-3
Session VI : METABOLISM AND QUALITY Chair : **Asaph Aharoni** *(Weizmann Institute of Science, Israel)*		
09:30-10:00	**Asaph Aharoni** – *Invited lecture* Solanum Steroidal Glycoalkaloids: from a Greasy Start to the Bitter End	SP-6
10:00-10:15	**Guillaume Bauchet** Metabolic genome-wide-association unravels trait architecture and natural variation in tomato primary and secondary metabolisms	OC-22
10:15-10h30	**José Luis Rambla** Variability in tomato fruit volatiles is interspersed within and across the tomato clade gene pool	OC-23
10:30-10:45	**Avichai Amrad** Evolution of pollination syndromes and scent production in Petunia	OC-24
10:45-11:15	*Coffee Break and Poster session*	
Session VII : SYSTEMS BIOLOGY AND MODELLING Chair : **Jocelyn Rose** *(Cornell University, US)*		
11:15-11:45	**Jocelyn Rose** – *Invited lecture* The tomato expression Atlas: A new platform for biological discovery with cell-type resolution	SP-7
11:45-12:15	**Sophie Colombié** – *Invited lecture* Modelling metabolic fluxes during tomato fruit development	SP-8
12:15-12:30	**Alain Tissier** Systems biology of tomato type VI glandular trichomes: insights into a metabolic cell factory	OC-25
12:30-12:45	**Iben Sorensen** High resolution characterization of the tomato fruit glycome	OC-26
12:45-14:15	*Lunch and Poster session*	
14:15-17:30	**PARALLEL SESSIONS**	

PARALLEL SESSIONS

	TO I : TOMATO Chair : Mondher Bouzayen	PO I : POTATO Chair : Christian Bachem	CO I : COFFEE Chair : Alexandre de Kochko / Dominique Crouzillat
	MAIN AUDITORIUM	**AMPHITHEATER N° F**	**AMPHITHEATER N° G**
14:15-14:45	**Mondher Bouzayen** - *Invited lecture-SP-9* The transcriptional regulatory network underlying fleshy fruit ripening: a case of the interplay between different hormone signally pathways	**Christian Bachem** - *Invited lecture-SP-10* Environmental and internal cues that regulate sexual and asexual reproduction in potato	**A De Kochko** / **D. Crouzillat -** *Invited lecture-SP-11* Aims and goals of the Arabica Coffee Genome Consortium (ACGC)
14:45-15:00	**Mohammad Irfan** –OC-27 Fruit Ripening Regulation of α-Mannosidase expression by the MADS Box Transcription Factor RIPENING INHIBITOR and Ethylene	**Gina M. Pham** –OC-35 Gene copy number and allele dosage in autotetraploid potato contribute to gene expression regulation	**Susan Strickler** –OC-44 Improving *Coffea* Genome Assembles with Long Read Data
15:00-15:15	**Julien Pirrello** –OC-28 Ethylene Response Factors involved in tomato fruit ripening and their connection to master regulators of the ripening process	**Marta Brylińska** –OC-36 *Phytophthora infestans* genetic diversity and effector expression in a single field experiment	**Simone Scalabrin** –OC-45 Progress report on the sequencing and assembly of the allotetraploid *Coffea arabica var*. Bourbon genome
15:15-15:30	**Flavia Krsticevic** –OC-29 Intronless tandem duplicated class I sHsp genes involved in *Solanum lycopersicum* (cv Heinz 1706) fruit ripening	**Norma C. Manrique Carpintero** –OC-37 Use of haploid populations to unravel the heterozygosity of autotetraploid cultivated potato	**David Sankoff** –OC-46 *Coffea*, *Rhazya*, and the evolution of the Gentianales
15:30-15:45	**Antonio J Monforte**–OC-30 Detection of QTLs involved in tomato fruit quality in a new genomic library of introgression lines from *Solanum pimpinellifolium* L	**Sjaak van Heusden** –OC-38 A diploid homozygous self-compatible inbred line in *S. tuberosum*	**Ray Ming** –OC-47 Integration of *C. canephora* into Arabica variety Catimor: a genomic view

	PE I : PEPPER/EGGPLANT Chair : Ilan Paran	SM I : SPECIALIZED METABOLISM Chair : Rob Last	PQ : PHOTOSYNTHESIS AND FRUIT QUALITY Chair : René Klein Lankhorst
	AMPHITHEATER N° H	**AMPHITHEATER N° A**	**AMPHITHEATER N° B**
14:15-14:45	**Ilan Paran** - *Invited lecture -SP-12* Improvement of pepper fruit quality by molecular breeding	**Robert Last** - *Invited lecture -SP-13* Small genetic changes, big phenotypic effects: the evolution of trichome specialized metabolism in Solanum and beyond	**Jeremy Harbinson** - *Invited lecture-SP-14* Photosynthesis as a target for improvement – can it be done and would it be useful?
14:45-15:00	**Lorenzo Barchi** –OC-51 A high quality eggplant (*Solanum melongena* L.) genome draft allows the mapping of phenotypic and metabolic QTLs	**Stefan Bennewitz** –OC-57 Using a backcross population to investigate the morphology and metabolism in type VI glandular trichomes in tomato	**Briardo Llorente** –OC-65 Light signaling pathways are recruited to adjust tomato fruit carotenoid biosynthesis to the progression of ripening by sensing chlorophyll contents
15:00-15:15	**Louise Chappell-Maor** –OC-52 MicroRNA156/7-mediated control of anthocyanin pigment accumulation in eggplant fruit peel	**Giovanni Giuliano** –OC-58 Concerted transcriptional-metabolic remodeling underlies the transition from green-fruited to red-fruited tomato species	**Himabindu Vasuki Kilambi** –OC-66 Role of Phototropin1 in Fruit Ripening
15:15-15:30	**Gaëtan Maillot** –OC-53 Toward a characterization of the pepper host resistance effect on the gene expression of the pathogenic *Phytophthora capsici*	**Pablo D. Cardenas** –OC-59 The AP2/ERF-type Transcription Factor GLYCOALKALOID METABOLISM 9 Regulates Cholesterol and Steroidal Alkaloid Biosynthesis in the Solanaceae	**María F. Cocaliadis** –OC-67 Enhancing Photosynthesis in tomato fruit to increase tomato fruit quality
15:30-15:45	**Martin Ganal** –OC-54 Development and characterization of a 19K Illumina Infinium genotyping array for pepper (*Capsicum*)	**Jamuna Risal Paudel** –OC-60 Reduced Steroidal Glycoalkaloid Levels Affects *Solanum tuberosum* Biotic Stress Resistance	**Livia Spicher**–OC-68 Response of plants to abiotic stress: a story of lipids

15:45-16:15	*Coffee Break*

	TO II : TOMATO Chair : Tohru Ariizumi	PO II: POTATO Chair : Christian Bachem	CO II : COFFEE Chair : Alexandre de Kochko / Dominique Crouzillat
	MAIN AUDITORIUM	**AMPHITHEATER N° F**	**AMPHITHEATER N° G**
16:15-16:30	**Lucio D'Andrea** –OC-31 A role for the Clp protease complex in chromoplast differentiation and carotenoid biosynthesis during tomato fruit ripening	**Parker Laimbeer** –OC-39 Residual Heterozygosity through cycles of Inbreeding in a Diploid Potato Population	**Alan Andrade** –OC-48 Genome Wide Association Study for Drought Tolerance and Other Agronomic Traits of a *Coffea canephora* Population
16:30-16:45	**Pierre Baldet** –OC-32 Vitamin C and Cell Wall Metabolisms in Tomato: GME a key actor of these interrelated pathways	**Csaba Hornyik** –OC-40 Small RNA regulation of potato tuber skin and flesh colour	**Lorenzo Del Terra** –OC-49 Identification of aquaporins in *Coffea arabica* L., gene expression study and correlations with plant water relations and hydraulics
16:45-17:00	**Chiaki Matsukura** –OC-33 Suppression of ADP-glucose pyrophosphorylase genes affects fruit skin thickness as well as fruit sugar and sugar phosphate contents in tomato (*Solanum lycopersicum* L.)	**Teresa Mosquera** –OC-41 Identification of candidate genes associated with quantitative resistance to late blight in Solanum tuberosum Group Phureja using association mapping	**Maud Lepelley** –OC-50 Diterpenes metabolism and related genes in coffee
17:00-17:15	**Atiyeh Kashaninia** –OC-34 Environmental Stresses affecting *Bemisia tabaci* Resistance in Tomato	**Jadwiga Śliwka** –OC-42 Diversity of *Fusarium spp*. associated with dry rot of potato tubers in Poland	
17:15-17:30		**Mark Taylor**–OC-43 Solanesol: Added value from Solanaceous waste	

	PE II : PEPPER/EGGPLANT Chair : Doil Choi	SM II : SPECIALIZED METABOLISM Chair : Rob Last	TB : TOBACCO Chair : Nikolai Ivanov
	AMPHITHEATER N° H	**AMPHITHEATER N° A**	**AMPHITHEATER N° B**
16:15-16:30	**Doil Choi** - *Invited lecture-SP-15* Multiple de novo genome sequences of hot pepper provide insights into species diversification in Capsicum spp.	**Marilise Nogueira** –OC-61 Tomato, a cell factory for the production of high value ketocarotenoids	**Nikolai Ivanov** - *Invited lecture-SP-16* Nicotiana genomics: from plants to genomes
16:30-16:45		**Victoria Gomez** –OC-62 An O-methyltransferase modifies accumulation of methylated anthocyanins in seedlings of tomato	
16:45-17:00	**Jaime Prohens** –OC-55 A strategy for broadening the genetic base of eggplant using wild relatives as donors of variation	**Ying Wang** –OC-63 Carotenoid biosynthesis and accumulation in fleshy fruits of *Lycium* L	**Corinne Mihri** –OC-69 Genomic changes generated in natural and synthetic *Nicotiana* allotetraploids: what do transposable elements tell us ?
17:00-17:15	**Pietro Gramazio** –OC-56 De novo transcriptome sequencing in four non-model species in genus Solanum (*S. incanum*, *S. aethiopicum*, *S. muricatum* and *S. caripense*): Analysis and molecular markers detection for breeding purposes	**Yellamaraju Sreelakshmi** –OC-64 Diversity in fruit color in the tomato clade-do we have the answers?	**Amanda Kozlo** –OC-70 Properties of *Nicotiana glauca* as a biorefining feedstock
17:15-17:30			**Natalia Carreno-Quintero** –OC-71 Development and evaluation of a potential bio-refining cascade for *Nicotiana glauca*

20:00	*Gala Dinner*

Thursday, October 29th

08:45-09:30	**Avi Levy** - *Keynote lecture* Crossover, mutagenesis and transformation – from randomness to precise breeding			KL-4
	Session VIII : NEW TOOLS FOR GENE DISCOVERY AND BIOTECHNOLOGY Chairs : **Antonio Granell** *(IBMCP-CSIC Valencia, Spain)*			
09:30-10:00	**Tohru Ariizumi** – *Invited lecture* Towards improved fruit set efficiency using tomato genetic resources			SP-17
10:00-10:30	**Diego Garcia-Orzaez** – *Invited lecture* Goldenbraid a multigene assembly platform for Solanaceae engineering and editing			SP-18
10:30-11:00	*Coffee Break and Poster session*			
	Session IX : BIOTIC STRESS I Chair : **Chuanyou Li** *(Chinese Academy of Sciences, China)*			
11:00-11:30	**Chuanyou Li -** *Invited lecture* Genetic dissection of systemin/jasmonate-mediated systemic defense signaling in tomato			SP-19
11:30-11:45	**Myluska Caro** Assessing the genetic variation of Ty-1 and Ty-3 alleles conferring resistance to Tomato yellow leaf curl virus in a broad tomato germplasm			OC-72
11:45-12:00	**Christos Kissoudis** Tomato responses to salt stress and powdery mildew combination: genetic resistance and the effect of salt stress intensity			OC-73
12:00-14:00	*Lunch*			
	Session X : BIOTIC STRESS II Chair : **Yuling Bai** *(Wageningen university, Netherlands)*		**Session XI : ABIOTIC STRESS** Chair : **Francisco Pérez-Alfocea** *(CEBAS-CSIC Murcia, Spain)*	
14:00-14:30	**Yuling Bai** *Invited lecture* Plant susceptibility genes in resistance breeding: promises and limitations	SP-20	**Francisco Perez Alfocea** *Invited lecture* Hormonal and metabolic regulation of abiotic stress responses in tomato	SP-21
14:30-14:45	**Nemo Peeters** Understanding the mechanisms of bacterial wilt disease for future sustainable resistance breeding in Solanaceae	OC-74	**Ernest Aliche** Carbon partitioning in potatoes under drought stress	OC-78
14:45-15:00	**Michela Appiano** Monocot and dicot MLO powdery mildew susceptibility factors are functionally conserved in spite of the evolution of class-specific molecular features	OC-75	**Marcin Pieczynski** New potato genetic elements involved in response to drought stress	OC-79
15:00-15:15	**Lei Wang** Identification of the Receptor for Bacterial Cold Shock Protein from Tomato	OC-76	**Elise Albert** Genotype by watering regime interaction in cultivated tomato: from phenotypes to genes	OC-80
15:15-15:30	**Katja Witzel** Comparative Proteomic Analysis of Tomato Roots Colonized by *Verticillium dahlia*	OC-77	**Anida Mesihovic** Regulation of pollen thermotolerance in tomato *(Solanum lycopersicum)*	OC-81
15:30-16:00	Closing of SOL 2015 Presentation of SOL 2016			

The 12th Solanaceae Conference

SOL2015

Abstracts

Lectures
Oral Communications

Monday, October 26th

KL-1

Towards understanding development and diversity of leaf shape

Tsiantis M and al

A key challenge in biology is to understand how diversity in organismal form is generated. Genetic analyses in model systems have identified key regulators that sculpt the body plans of metazoa and seed plants. However, less is known about how the action of such regulators produces particular organ shapes, and how the balance of conservation versus divergence of fundamental developmental pathways generated the tremendous morphological diversity of multicellular eukaryotes. One impediment to answering these questions is the relative paucity of experimental platforms where genetics and developmental biology tools and can be combined effectively to study causal links between genotypic variation and phenotypic evolution in a genome-wide, unbiased fashion, and at different evolutionary scales. To circumvent these problems we developed the Arabidopsis thaliana relative Cardamine hirsuta into a versatile system for studying morphological evolution. We are investigating the morphogenetic mechanisms through which morphology evolved in these species, resulting in simple, undivided leaves in A. thaliana and dissected leaves with distinct leaflets in C. hirsuta. This presentation will discuss our progress towards understanding the genetic pathways that specify dissected versus entire leaf shapes and that regulate the number, position and timing of leaflet production.

OC - 1

Stamen development in tomato – jasmonates control ethylene action in anther dehiscence and pollen release

Dobritzsch S[a], Schubert R[a], von Sivers L[a], Kopka J[b], Hause B[a]

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Jasmonates are well known plant signaling components required for stress responses and development. A prominent feature of jasmonate biosynthesis or signaling mutants is the loss of fertility. The tomato mutant *jai1-1* exhibits female sterility with additional severe effects on stamen and pollen development. Its senescence phenotype suggests a function of jasmonates in regulation of processes known to be mediated by ethylene. To test the hypothesis that ethylene involved in tomato stamen development is regulated by jasmonates, a temporal profiling of hormone content, transcriptome and metabolome of tomato stamens was performed using wild type and *jai1-1*.
Comparative transcriptome analyses revealed a diminished expression of genes involved in pollen nutrition at early developmental stages of *jai1-1* stamens, but an enhanced expression of ethylene-related genes at late developmental stages. The latter coincides with an early increase of the ethylene precursor ACC and a premature pollen release from stamens, a phenotype similarly visible in an ethylene overproducing mutant. This suggested an essential role of jasmonates in the temporal inhibition of ethylene production to prevent premature desiccation of stamens. To test this, *jai1-1* was crossed with *NeverRipe*, an ethylene insensitive mutant. Indeed, the double mutant showed a complementation of *jai1-1* phenotype in terms of dehiscence and pollen release.

OC - 2

The circadian clock of cultivated tomato has been slowed down during domestication

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The circadian clock is an endogenous timekeeper regulating many important aspects of plant physiology and development, including key agricultural traits in crop plants. Additionally, natural variation in circadian rhythms is important for local adaptation. However, quantitative modulation of circadian rhythms due to artificial selection has not yet been reported. By analyzing circadian leaf movements of a variety of wild and cultivated tomato accessions we demonstrate that the circadian clock of cultivated tomato (*Solanum lycopersicum*) has been slowed down during domestication. Quantitative trait locus (QTL) analyses reveal allelic variation of the tomato homolog of the Arabidopsis gene *EMPFINDLICHER IM DUNKELROTEN LICHT1* (*EID1*) to underlie the delayed circadian phase of cultivated tomato. Notably, the genomic region surrounding *EID1* shows signatures of a selective sweep indicating that it has been under positive selection. Finally, we find that the cultivated allele of *EID1* enhances tomato performance specifically under long day photoperiods suggesting that humans selected slower circadian rhythms to adapt the cultivated species to the long summer days it encountered as it was moved away from the equator.

OC - 3

How to make it branched: a recently evolved alternative splice site in BRANCHED1a controls plant architecture in potato

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Amplification and diversification of transcriptional regulators that control development is a driving force of morphological evolution. In addition, a major source of protein diversity is alternative splicing. The mechanisms and timing of intron evolution nonetheless remain unclear, and the functions of alternative splicing-generated protein isoforms are rarely studied. In Solanum tuberosum, the BRANCHED1a (BRC1a) gene encodes a TCP transcription factor that controls lateral shoot outgrowth. Here we report the recent evolution in Solanumof an alternative splice site in BRC1a that leads to the generation of two BRC1a protein isoforms with distinct C-terminal regions, BRC1aLong and BRC1aShort, encoded by unspliced and spliced mRNA, respectively. The BRC1aLong C-terminal region has a strong activation domain, whereas that of BRC1aS lacks an activation domain and is predicted to form an amphipathic helix, the H domain that prevents protein nuclear targeting. BRC1aShort is thus mainly cytoplasmic, while BRC1aLong is mainly nuclear. BRC1aLong functions as a transcriptional activator, whereas BRC1aShort appears to have no transcriptional activity. Moreover, BRC1aShort can heterodimerize with BRC1aLong and act as a dominant negative factor; it increases BRC1aLong concentration in cytoplasm and reduces its transcriptional activity. This alternative splicing mechanism is regulated by external and hormone factors that control branching. The evolution of a new alternative splicing site and a novel protein domain in Solanum BRC1a led to a multi-level mechanism of post-transcriptional and post-translational BRC1a regulation that effectively modulates its branch suppressing activity in response to environmental and endogenous cues. (In press in Current Biology,

OC - 4

Boundaries shape tomato shoot architecture and initiate new meristems

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Boundaries, which are established and maintained in different regions of the plant body, have diverse functions in development. Firstly, they separate different cell groups, for example the differentiating cells of a leaf primordium from the pluripotent cells of the apical meristem. In compound leaved species, like tomato, boundaries separate young leaflets from each other and from their connecting structure, called rachis. Recent experiments have shown that boundary zones between the meristem and leaf primordia show similar properties as boundaries between leaflets. They are characterized by a low rate of cell divisions and specific patterns of gene expression. We identified *Potato leaf*, the closest paralog of the shoot branching regulator *Blind*, as a key regulator of leaf dissection in tomato (Busch et al., 2011). A combination of loss-of-function alleles of *Potato leaf* and *Goblet*, a known leaf complexity regulator, results in simple leaves. Secondly, boundary zones initiate new meristems, which requires a low level of the plant hormone auxin (Wang et al., 2014). Disruption of polar auxin transport compromises axillary meristem formation. New meristems do not only initiate in the meristem-to-leaf primordium boundary, but also in the distal leaflet boundary (DLB) of tomato leaves (Rossmann et al., 2015). Initiation of these ectopic meristems is dependent on activities of the well-known branching regulators *Goblet* (*Gob*) and *Lateral suppressor* (*Ls*), which act in hierarchical order. Ectopic meristem formation at the DLB is also observed in other seed plants, like *Cardamine pratensis*, indicating that it is part of a widespread developmental program.

OC - 5

A time to flower: Insights into the photoperiodic regulation of flowering in Tobacco

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Regulation of flowering-time is a very important trait, contributing to yield and quality in many crop-species. Tobacco (*Nicotiana tabacum*) is typically considered day-neutral for flowering, but is particularly interesting as it arose from a hybridisation between two ancestral species that display facultative long-day and facultative short-day flowering (*N. sylvestris* and *N. tomentosiformis* respectively). Harig et al., (2012) previously identified 4 tobacco *FT* genes; 3 of which were shown to suppress flowering (*NtFT1*, *NtFT2* and *NtFT3*), and one of which was shown to induce flowering (*NtFT4*). Using a *de novo* assembly of the tobacco genome, we uncover an additional repressor - homeologus to *NtFT1* - that shows no evidence of expression and contains a stop codon in the first exon of the gene; a mutation which appears to be conserved from the ancestral *N. sylvestris* genome. Additionally, we show that tobacco actually has 3 copies of the floral-inducing *NtFT4* gene; with homeologus *N. tomentosiformis* and *N. sylvestris* genes, the second of which is duplicated approximately 75kb upstream. Four further FT genes were also identified, likely having floral induction function based on a conserved set of three amino acids identified in sugar beet (*Beta vulgaris*), as well as the absence of a potentially *Solanacea*-wide repressor-associated domain in the same region of the protein. We investigate the expression of these genes, and others in the canonical photoperiodic flowering pathway, to provide a better understanding of the regulation of flowering in *Nicotiana*, in particular considering the impact of genome duplication on this complex trait.

SP - 1

Returning to domestication to revitalize crop improvement: engineering the florigen system.

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Successful plant breeding involves a combination of intuition, experience, and large-scale, time-consuming selection schemes. A major challenge in modern agriculture is to develop new approaches that can expedite breeding and make its outcomes more predictable. The last decade of studies on crop domestication has pointed to a recurring theme: selection of genetic variation in the universal florigen flowering system, which includes the flower-promoting hormone florigen and antagonistic family members. Prominent examples include continuous flower production in strawberries and roses, biennial flowering in sugar beet, adapted flowering in barley, and the introduction of a bushy determinate growth habit for bean, soybean, and tomato, which led to large-scale field production. Yet, these successes are all based on rare naturally occurring mutations that may not be providing optimal agronomic performance. I will discuss our recent work on translating discoveries on genetic and molecular mechanisms underlying florigen-dependent flowering in tomato to engineer fine-tuned and optimized plant architecture and yield. I will also present a new CRISPR-based approach we are developing to create novel quantitative genetic variation that has the potential to invigorate breeding in all crops.

SP - 2

Using molecular diversity in wild tomatoes for inferences on evolution and speciation

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Patterns of nucleotide polymorphism across genomes are expected to be shaped by both negative and positive selection, genetic drift, and differences in recombination and mutation rates. We have added to the growing number of empirical studies in plants by transcriptome sequencing of several wild tomato species (*Solanum* section *Lycopersicon*) sampled with range-wide coverage, yielding data for thousands of expressed genes. This clade spans a diversity of mating systems from obligate outcrossing to highly self-fertilizing, generating a range of effective recombination rates across species that should have implications for linked selection. In concert with differences in gene density and physical recombination across genomic regions, I will present evidence on levels of purifying selection across species as well as inferences on linked selection. In a second line of inquiry, we have studied hybrid seed failure, an important postzygotic barrier to interbreeding among species of wild tomatoes and other angiosperm groups. Our pilot molecular data concern the closely related *S. peruvianum* and *S. chilense*; hybrid crosses between these species yield very high proportions of inviable seeds due to endosperm failure and arrested embryo development. Based on seed size differences in reciprocal hybrid crosses and developmental evidence implicating endosperm failure, we hypothesized that (perturbed) genomic imprinting might be involved in this strong postzygotic barrier. Consequently, we surveyed endosperm transcriptomes obtained via laser-assisted microdissection of developing seeds representing both intra- and interspecific pollinations. I will discuss our evidence for fundamentally changed maternal transcript proportions in (failing) hybrid endosperm.

OC - 6

Genomic variation in tomato, from wild to cultivated

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Genomic variation of 1008 accessions of *Solanum pimpinellifolium* (SP) and *S. lycopersicum* (SL) was analyzed with the SolCAP genotyping platform. The genotypes for the fruit shape and weight related genes were also obtained. SP was clearly split in the PCA analysis in two distinct groups, one that covered Peru and Southern Ecuador and another located in Northern Ecuador. Most of non-commercial *S. lycopersicum* var. *cerasiforme* (SLC) samples were likely to be semi-domesticated intermediates between the wild and cultivated species, while many commercial cherry tomatoes were dispersed in the PCA with the SPxSLL hybrids. The cultivated alleles for most of the fruit related genes were already found in Ecuadorian and Peruvian SLC. Some of these SLC accessions were collected in markets as cultivated tomatoes. These evidences suggested a first step in the tomato domestication in Peru and Ecuador followed by a further increase in fruit size in Mesoamerica. The genetic diversity indexes showed that the bottleneck in the tomato domestication occurred in the Andean to Mesoamerica migration. The PCA showed that the vintage and modern tomatoes derived mainly from Mesoamerican SLL. A rarefaction analysis showed only a moderately increase in the contemporary SLL variability. This increase is compatible with only few introgressions from wild materials added to the vintage tomato gene pool to create the contemporary materials.

OC - 7

Working out the history and the biogeography of Old World "spiny solanums" (*Solanum* subg. *Leptostemonum*): the key contribution of the overlooked Tropical Asian species

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A giant among the flowering plants, Solanum L. (c. 1500 species) has a cosmopolitan distribution and numerous plants of global agricultural importance (e.g., potato, tomato, aubergine). Within Solanum, the spiny solanums clade forms the most species-rich major lineage (c. 450 species) (Vorontsova & al., 2013). In contrast to their New World relatives, Old World spiny Solanum have received little attention. The Asian taxa in particular have never been revised in their entirety since Dunal (1852) and have been sparsely sampled in all phylogenetic analyses to date. This has significantly impeded understanding of Solanum's evolutionary history. Based on sampling from Africa and Australia, Old World taxa have been characterized as a monophyletic group (Stern & al., 2011; Vorontsova & al., 2013). To test this hypothesis, we are clarifying Asian spiny solanums species delimitation and building broad molecular sampling. Our results (Aubriot & al., in prep.), based on a representative sampling of Tropical Asian spiny solanums and 5 DNA markers show that these taxa do not all resolve with other Old World species, some being instead members of New World clades. Our data suggest at least three independent introductions from the New World, thus shedding new light on the biogeography of spiny Solanum. We are now enlarging sampling and using NGS approaches in order to further clarify these patterns and fill this major gap in knowledge of Solanum phylogeny.

OC - 8

Taxonomic application of the leaf anatomy in species of *Solanum* clades *Gardneri* and *Thomasiifolium*

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Leaf anatomical studies with a multivariate analysis were conducted to evaluate its taxonomic significance in two clades of Solanum subg. Leptostemonum1: the Gardneri clade, with seven species (S. agrarium Sendtn., S. gardneri Sendtn., S. polytrichum Moric, S. schomburghii Sendtn., S. stenandrum Sendtn., S. talarense Svenson and S. tetramerum Dunal); and Thomasiifolium clade, with four species (S. buddleifolium Sendtn., S. paraibanum Agra, S. rupincola Sendtn. and S. thomasiifolium Sendtn.). For each species, three leaves were removed from five individuals, which were submitted to paradermic sections of the epidermis, and cross sections of the mesophyll, midrib, and petiole, following the usual techniques of plant anatomy. Measures of the length and width of stomata were taken, and T-test was applied. As results, all species showed anisocytic stomata, dorsiventral mesophyll, angular collenchyma, and bicollateral vascular system pattern. Species of the Gardneri clade have stomata slightly above of the epidermal cells, and plane-convex petiole; the clade thomasiifolium showed stomata at the level of the epidermis, and biconvex petiole, winged. The hierarchical clustering analyzes also showed the formation of two major groups, corresponding to the clades Gardneri (A) and Thomasiifolium (B). The Gardneri clade obtained good bootstrap index, but relations between S. schomburghii and S. polytrichum and other species of the group are unclear. The Thomasiifolium clade showed a clear distinction between paraibanum-rupincola and thomasiifolium-buddleifolium. The leaf anatomy done in this study corroborates the date of the phylogeny of Gardnerii and Thomasiifolium clades, and can be a tool to support its taxonomy (Financial support: CAPES, CNPq and FACEPE)

OC - 9

Evolutionary analysis of Petunia genomes

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Petunias are one of the most popular bedding flowers. They are widely distributed along the whole world as a part of the spring urban landscapes from small towns to big cities. Nevertheless they have humble origin. Garden petunias (Petunia hybrida) come from the hybridization of white flower petunias (P. axillaris) and purple flower petunias (probably P. inflata) from Brazil and Uruguay. Beyond their ornamental uses, petunias have been a model to study several biological processes such as flower development, anthocyanin biosynthesis, scent production, pollination syndrome, self-incompatibility and transposons dynamics. In this work, we present the evolutionary analysis of two Petunia genomes (P. axillaris N and P. inflata S6), its comparison with other Solanaceae, some insights in the complex origin of the cultivated petunias based in three P. hybrida lines (Mitchell, R29, R143) transcriptomes and updates, in the genomic framework, of the genes involved in adaptation (e.g. circadian clock) and reproduction (e.g. self-incompatibility). 35,812 and 39,408 genes have been annotated for the 1.26 (P. axillaris) and 1.29 Gb (P. inflata) genome assemblies respectively. The analysis of the genome synteny and gene synonymous changes (Ks) distributions supports the paleohexaploidy event described in the analysis of the tomato genome.

OC - 10

Genome reduction suggests large dispensable genome and adaptive role for copy number variation in asexually propagated *Solanum tuberosum*.

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Clonally reproducing plants may have potential to retain a significantly greater mutational load than some sexually reproducing species, due to an absence of meiosis in most generations. To investigate this possibility, we examined the breadth of genome-wide copy number variation (CNV) in a panel of 12 monoploid/doubled monoploid clones generated androgenetically from diploid landrace populations of potato (*Solanum tuberosum*), a heterozygous asexually propagated plant. As rare instances of purely homozygous potato clones, these provided an ideal set for determining the degree of structural variation tolerated by this species, and deriving its minimal gene complement. Copy number variation in this relatively limited set of germplasm was more extensive than several sexually reproducing plant species, impacting ~30% of assembled, non-repetitive sequence in the potato genome, on par with levels of variation observed in maize using larger sample sizes. Many potato genes appear subject to duplication or deletion, revealing the highly heterogeneous nature of the potato genome. Dispensable genes often show limited transcription or a recent evolutionary history, whereas deletion was less frequent in genes whose orthologs are conserved among angiosperms. Association of CNV with plant adaptation was highlighted by enrichment in gene clusters associated with environmental response and species-specific expansions of stress-related gene families (SAURs, methylketone synthase I). The unique germplasm employed in this study suggested a significant impact of CNV in a species with asexual reproductive habit, and a role for CNV driving adaption through evolution of key stress pathways.

SP - 4

Recent advances in development of tools for potato genetics and genomics

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The Potato Genome Sequencing Consortium (PGSC) published the 'DM' genome in 2011, and a much improved version (v4.03) of the genome pseudomolecules in 2013. The availability of the genome sequence has facilitated development of extensive tools for genetic analysis in this important crop. One of these is the SolCAP 8303 SNP array which has been used extensively for trait analysis in segregating populations and association panels. We have recently published a dense linkage map of a diploid cross as well as an analysis of tuber shape and eye depth. Ongoing analysis of this cross focuses on other agronomic traits as well as resistances to late blight and Potato Virus Y. Genotyping by sequencing (GBS) approaches are also a cost-effective method for genotyping in potato and we have used this approach for both linkage and QTL analysis in biparental populations and association mapping in variety panels. Results from these and other analyses suggest that association mapping is a very effective method for the detection of marker trait associations in tetraploid potato. In performing this type of study a careful assessment of population structure is required to avoid spurious associations. Various models have been examined, insights from which will be presented. A further development is the recent construction of an exome wide capture platform that is being used for various mapping studies, as well as the study of diversity and evolution in potato. Results from some recent analyses will be presented.

OC - 11

Assessment of factors affecting genomic selection in populations of tomato

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Marker-assisted selection is traditionally used to improve traits in crops. However, it requests very long cycles selection and still unable to capture loci of minor effect. Genomic selection (GS), a new tool for selecting elite plants in a breeding program by predicting the performance of traits of interest in application of statistical model, is an exciting alternative approach. GS has the potential to catch the effect of all markers underlying the genetic architecture of the studied traits.
In this context, we aimed at evaluating the use of GS into tomato to evaluate the prediction accuracy for 25 traits related to the fruit quality for three different type of population (RIL, MAGIC and GWA panel). We conducted a cross validation approach while estimating the relative weight of parameters (training and testing populations sizes, genetic composition, predictive statistical models, markers density, heritability of the trait as well as the type of population) onto the prediction accuracy.
Our results demonstrated that GS seems very powerful at predicting phenotypes values with accuracies ranging from 0.12 to 0.84 in the panel. Precisely, we conclude that (1) optimizing the training set the better the predictions are (+13% on average) (2) the larger the heritability of the trait value is, the better the predictions are (3) the statistical prediction models perform very similarly and (4) the optimal markers density is depending of the population LD.

Thus, applying GS in tomato seems very promising and could be used tomato breeding programs and produce high-quality varieties.

OC - 12

Genomics-assisted selection of *Solanum chilense* introgression lines for enhancing drought resistance in tomatoes

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Breeding for improved water use efficiency, and ability to give high yields where water availability is poor, are important traits for tomato. *Solanum pennellii*, *S. chilense* and *S. sitiens* are three key tomato wild relatives that are found in arid areas and have the greatest adaptation to water-limited environments. However, good genetic and genomic resources only exist for *S. pennellii*, where well-established and highly successful strategies have been employed to exploit allelic variation. We aim to develop genetic and genomic resources to allow exploitation of the adaptive traits for *S. chilense* and *S. sitiens,* both allogamous, self-incompatible (out-breeding) species. We have obtained 231.5 and 171.8 Gbp Illumina data for *S. chilense* LA1972 and *S. sitiens* LA1974, respectively, and *de novo* genome assembly is underway.

A hybrid between *S. chilense* LA1972 and cultivar VF36 was obtained by embryo rescue and introgression line (IL) and in-bred backcross line (IBL) populations are being constructed by crossing to the Indian cultivar "Kashi Amrit". We will select a set of 384 marker SNPs across the genome for use in population development. Populations will be used to screen for QTL for drought resistance and water use efficiency. In addition, a bridging line derived from *S. arcanum, S. chilense* and *S. lycopersicum* (SB5) is being backcrossed to MicroTom, and material will be used to select for resistance to wilting under water deficit.

OC - 13

Understanding hybrid seed failure in wild tomatoes: phenotypic and transcriptomic signatures

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Hybrid seed failure is a common reproductive barrier between plant species. For plant breeders, it represents a major obstacle to introgression of desirable traits from wild to domesticated species. Postzygotic barriers to hybridization have been well-documented among wild tomato species, and histological work showed that endosperm failure is the main cause of seed abortion. Based on an updated phylogeny of the tomato clade, we addressed hybrid seed failure between three taxa, namely *Solanum peruvianum*, *S. chilense* and *S. arcanum* var. maranon). We characterized mature seed size and viability in a large number of crosses and conducted endosperm-specific RNAseq experiments using six reciprocal crosses within and between the three taxa. The crossing design was chosen to detect expression pattern differences with regard to overall and parent-of-origin- specific expression (i.e. imprinting). Reciprocal interspecific crosses involving *S. peruvianum* yielded no viable seeds and were classified as having a 'strong' barrier. Crosses between *S. chilense* and *S. arcanum* var. maranon were characterized by variable levels of seed viability as well as asymmetric outcomes in some reciprocal crosses, here classified as a 'soft' barrier. Seed size was significantly reduced with *S. peruvianum* in the maternal role in hybrid crosses, compared to crosses within this species. Transcriptome analyses shows drastic expression changes when comparing intraspecific crosses –with normally developing endosperm– and among-species crosses with abnormal endosperm. We propose imprinting disturbance as a mechanism contributing to hybrid seed failure, but ongoing work will further characterize molecular pathways possibly involved in this reproductive barrier and its variability.

OC - 14

Genebanks and Genomics: how to interconnect data from both communities?

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How will genebanks use and provide access to genomics data and how will genomics information resources give access to genebank data and material? Herein, we discuss a possible solution that allows making curated passport and C&E data online available on the genebank side and sequences and their annotations on the genomics data provider side, including public resources such as NCBI databases (Anguita et al., 2013) and UniProt (Redaschi and Uniprot Consortium, 2009). This approach will allow to interconnect these data; a genebank can provide access to the annotated sequences or search for allelic variants for specific genomic regions within its genebank material; a genomic database can give access to the details about the origin of the accessions, or the phenotypic data available at the genebank. This interconnection is based on semantic web technology, an established framework of the World Interconnecting genebank and genomic data Wide Web Consortium (W3C, 2013).

OC - 15

In silico and empirical optimizations of ddRAD-Seq for tomato genomics, genetics, and breeding

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Double-digest restriction site-associated DNA sequencing (ddRAD-Seq) enables high-throughput genome-wide genotyping with next-generation sequencing technology. We designed and demonstrated a workflow for *in silico* and empirical ddRAD-Seq analysis in tomato, as follows: 1) *in silico* prediction of optimum restriction enzymes from the reference genome; 2) verification of the prediction by empirical ddRAD-Seq of four restriction enzyme combinations; 3) establishment of a bioinformatics pipeline for high-confidence single nucleotide polymorphism (SNP) calling; and 4) validation of SNP accuracy by construction of genetic linkage maps. This ddRAD-Seq pipeline could help accelerate genetics, genomics, and molecular breeding in both model and non-model plants, including tomato.

KL - 2

Genetic factors influencing tomato fruit ripening

James Giovannoni et al

Tomato (Solanum lycopersicum) is a tractable and efficient model for fruit development, storage quality and nutrient accumulation, in addition to being a vegetable crop of increasing production, consumption and culinary importance the world over. Diverse, painstakingly collected, intricately designed, well characterized and freely available germplasm resources, combined with efficient transformation and a high quality genome sequence have accelerated the pace of tomato biology with practical implications to crop improvement. Out lab explores the function of ripening transcription factors underlying fruit ripening mutations including those altered in the rin, nor, and u mutations defining fruit development roles for the MADS, NAC and GLK transcription factor families, respectively. Mining of these families provided additional genes effecting fruit development and ripening characterized in transgenic tomato plants. Additional regulators have been uncovered via examination of fruit quality QTLs and genes associated with ripening based on expression profiles during development and/or in specific maturing fruit tissues. Other researchers have identified and carefully characterized additional critical transcriptional regulators that add greatly to our understanding of ripening control such as (but certainly not limited to) the FUL1/2, CNR and AUX/ARF genes. Perhaps not surprisingly, many of these regulators have Arabidopsis counterparts shown to govern hormone biology and silique development. Genome enabled analysis of fruit development further indicates that transcriptional control intersects with changes in the epigenome. An overview of the diverse genetic regulators of fruit ripening in tomato will be presented, with examples of practical value and instances of leveraging tomato discoveries toward insights pertaining to the ripening and shelf-life of other fruit crops.

SP - 5

DNA-dependent fruit growth in tomato: endoreduplication and the karyoplasmic ratio theory.

Christian Chevalier

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Fleshy fruit species such as tomatoes are important because of their nutritional and economic value. Several stages of fruit development such as ovary formation, fruit set and fruit maturation have already been the subject of several developmental studies. However fruit growth *per se* has been much less addressed. Fruit growth like all plant organs depends upon the developmental processes of cell division and cell expansion. The activity of cell divisions sets the number of cells that will compose the fruit; the cell expansion activity then determines its final size. Among the various mechanisms that may influence the determination of cell size, endopolyploidy by the mean of endoreduplication, *i.e.* genome amplification in the absence of mitosis, appears to be of great importance in fleshy fruits. In tomato fruit, endoreduplication is associated to DNA-dependent cell expansion: cell size can reach spectacular levels such as hundreds of times its initial size (e.g. >0.5 mm in diameter), with as much as an 8-fold increase in nuclear DNA content (up to 512C, a DNA content rarely encountered in other plant species). Using tomato fruit development as a model, our recent investigations combining the use of flow cytometry, cellular imaging and molecular analyses has provided new data in favor of the long-standing karyoplasmic ratio theory, stating that cells tend to adjust their cytoplasmic volume to the nuclear DNA content. By establishing a highly structured cellular system where multiple physiological functions are integrated, endoreduplication does act as a morphogenetic factor supporting cell growth during tomato fruit development. In the context of plant breeding, deciphering the mechanisms controlling fruit growth, in particular those connecting the process of nuclear endoreduplication, the regulation of cell size and final fruit size and composition, will offer a valuable knowledge to understand better the establishment of fleshy fruit quality traits.

OC - 16

Engineering the florigen pathway in tomato

Soyk S, Park S, Brooks C and Lippman ZB

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Reproductive success and agricultural productivity is highly dependent on plant architecture, which is shaped by the activity of small groups of stem cells in shoot meristems. When environmental and endogenous conditions favor reproduction, vegetative meristems transition to a reproductive phase and give rise to reproductive branches – inflorescences – that bear flowers, fruits, and seeds. In perennial plants such as tomato, the reproductive transition causes meristems to mature into a flower, but not before new meristems are formed to continuously give rise to new shoots and flowers. Key regulators of this cycling between meristem termination and renewal are the flowering hormone florigen (encoded by *SFT, SINGLE FLOWER TRUSS*) and its antagonist anti-florigen (encoded by *SP, SELF PRUNING*), which act together in a dose-dependent manner to establish a ratio of opposing flowering signals that ultimately determines plant architecture and flower production. We recently characterized a toolkit of chemically-induced mutations in the florigen pathway that allowed modulation of florigen:anti-florigen ratios to fine-tune meristem maturation, shoot architecture, and yield. Here, we report on our expansion of this toolkit with naturally-occurring as well as CRISPR/Cas9-engineered alleles of thus far uncharacterized components of the tomato florigen pathway. By understanding at the molecular level how these new components integrate in the florigen pathway, we aim to define genetic and molecular principles underlying meristem maturation that can be exploited to improve agriculture beyond tomato.

OC - 17

Over-expression of SlyMIR159 alters flower development and promotes parthenocarpic fruit formation in tomato

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Fruit set, defined as the shift from the quiescent ovary to fast-growing young fruit, is a key process for fruit production in flowering plants. Research related to development are focused mainly on flesh fruits due to their importance on human diet. The fruit set and seed formation depends on the successful completion of pollination which triggers hormone production. Parthenocarpy, is fruit set in the absence of fertilization, and is potentially a desirable trait for many commercially grown fruits. It has been shown that hormones may act in parallel with transcription factors during fruit set, thus stressing the importance of the control of these genes along fruit development. Interestingly some of these genes are post-transcriptionally regulated by microRNAs (miRNAs). In this work, explored the possible role of microRNA159/*GAMYB* pathway during fruit set. We initially performed *in situ* hybridization experiments in pre-anthesis ovaries from tomato (cv Micro-Tom) with miR159 and *SlyGAMYB1* probes. The results showed that these genes are expressed during ovary development, mainly in developing ovules and pollen primordia. We then generated several independent transgenic lines overexpressing the *SlyMIR159* (named OE-S*lyMIR159*) and also transgenic lines transformed with an empty binary vector as a control. Interestingly, all transgenic OE-S*lyMIR159* plants showed flower modification and parthenocarpic fruits. Moreover, when OE- *SlyMIR159* ovaries were pollinated with pollen from Micro- Tom and other cultivars, grown fruits were also parthenocarpic. These results indicate that microRNA159/*GAMYB* pathway is relevant for fruit set, probably by interacting with other genetic pathways and hormones (such as auxin and gibberellin) during fruit set.

OC - 18

The coordinated pattern of cell division and cell expansion during fruit set and fruit growth in tomato pericarp

Cynthia Deluche, Nathalie Frangne, Catherine Cheniclet, Christian Chevalier, Jean-Pierre Renaudin

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We report a quantitative evaluation of cell size and cell number at the level of each cell layer of the ovary and fruit pericarp in cherry tomato cv. Wva106, from the flower stage before pollination to the end of the fruit growth phase. Cell expansion and cell division increased significantly in specific cell layers after anthesis as early as at 1DPA. The central mesocarp cells showed significant expansion at the same time as the three exocarp cell layers resumed mitotic activity with some indication of synchrony. The rate of these events shifted from low to high at fruit set, from *ca.* 3 DPA. Throughout the fruit growth phase, each of the 8 cell layers present in ovary wall at anthesis showed very distinct patterns of growth associating cell division and cell expansion to various extents. The cell number increased 20-fold up to 20 DPA. Cell expansion occurred in all cell layers and the mean volume of one pericarp cell increased 100-fold from anthesis to 20 DPA, with a range of values from 5-fold for outer epidermal cells to 2200-fold for central mesocarp cells. As a whole, the pericarp volume increased 2000-fold within 20 DPA, of which 17 % was accounted for by the increase of cell number and 83 % by the increase of cell volume. These data are discussed with regards to pericarp growth pattern, to the targets of fruit set-inducing signals and to the function of endoreduplication.

OC - 19

Active DNA demethylation controls tomato fruit ripening

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In plants, cytosine DNA methylation is a reversible epigenetic mark regulating gene expression and genome stability, which can be actively removed by bifunctional DNA glycosylase-lyases, the so-called DEMETER-like DNA demethylases (DMLs). In *Arabidopsis thaliana*, active DNA demethylation plays a critical role in the maternal imprinting and endosperm demethylation, but none of these functions appears to be essential for development in this species. We have now initiated the study of DMLs in the Tomato. Here we present evidence of a direct cause and effect relationship between active DNA demethylation mainly mediated by the tomato DML (SlDML2) and fruit ripening. SlDML2 knockdown results in ripening inhibition via hypermethylation and repression of the expression of genes encoding ripening transcription factors and rate-limiting enzymes of key biochemical process such as carotenoid synthesis. To define how SlDML2 affect fruit ripening at the genome wide scale, a global gene regulatory network associated with active DNA demethylation will now be established, combining metabolome, transcriptome analyses with the study of the genome wide distribution of methylation. So far, the results are consistent with DNA demethylation being necessary for tomato fruit ripening to occur, and also suggest additional roles on tomato flower and leaf development.

OC - 20

"Rocky" – A novel delayed ripening pepper mutant

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The pepper (Capsicum annuum L.) fruit is a true berry with diverse shapes and colors. During ripening, categorized as "non-climacteric", it undergoes dramatic changes in color, texture and content. . In a blocky pepper breeding population, a mutant was discovered that was characterized by an extreme delay in fruit ripening.. Instead of ripening the mutants' fruits exhibited exceptional increase in the fruits' firmness, and hence it was named "rocky". In addition, the rocky seeds do not germinate under standard conditions. However, the mature embryos can be grown to fully developed, normal appearing plants, when rescued from the seed coats and grown on culture media plates. A series of progeny tests with pepper plants from various genetic backgrounds revealed that 25% of the offspring of heterozygous parents were mutants indicating that rocky is a recessive single locus mutation. Examination of the common manifestations that accompany the pepper fruit's ripening, such as: color change, softening, decreased water content, decreased pH and increased sugar's concentrations revealed that whereas the normal fruits ripen, the rocky fruits retain the attributes of fruits similar to those of the un-ripe fruits. Examination of rocky seeds' size (surface area) showed that it was significantly larger than that of normal ones. In addition, endosperm accumulation in these seeds is impaired and the rocky embryos developed significantly later than normal ones. This is the first report about a ripening mutant of a non-climacteric fruit and its relatedness to tomato will make it a powerful tool for fruit ripening studies.

OC - 21

Cloning of a gene underlying a major texture QTL in tomato

Graham B. Seymour[a] Selman Uluisik[a], Charles Baxter[b], Daniel Rickett[b], : Jocelyn K.C. Rose[c] and Don Grierson[a]

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Softening in tomato, involves cell wall remodelling and changes in cuticle properties. The precise mechanism has been the subject of decades of research, but has remained elusive. We will present fine mapping of a gene underlying a major fruit texture QTL in tomato, identified using the S. pennellii introgression lines. The QTL candidate gene has been silenced in transgenic plants. The transgenic fruits are substantially firmer than the controls at the red ripe stage. Fruit colour, sugar content and volatile emissions were similar in the transgenic and control lines and RNA-Seq analysis indicated that silencing of the QTL candidate gene had limited effect on other ripening-related changes in gene expression. The effects are therefore specific to fruit softening. Cell wall analysis of the transgenic fruits has revealed information on the mechanistic basis of the texture effects and these data will be described in the presentation. This work indicates that it is possible to influence fruit firmness in the absence of a detrimental impact on other aspects of fruit quality and provide a strategy for enhancing shelf-life in tomato while maintaining excellent quality.

Integration of high-throughput data in models of plant metabolism

Zoran Nikoloski et al

Metabolic reactions comprise functional networks that are capable of bearing flux. Fluxes of metabolic reactions influence growth and other cellular tasks, and can be regarded as integrated outcomes of transcription, translation, and their regulation. Therefore, inference, prediction, and comparison of reaction fluxes allow insights in the molecular mechanisms that shape cellular functionality. However, fluxes are not measured directly, but are inferred by integrating other measurable quantities in models of metabolism. Recent metabolic modeling interests have shifted from small pathways (e.g., Calvin-Benson cycle) towards investigations of genome-scale compartmentalized tissue/organ-specific models. The constraint-based modeling framework has been successfully applied for flux prediction and comparison on a genome-scale level. Here, I will present recent computational methods for integration of (time-resolved) high-throughput data sets in large-scale models of photosynthetic organisms which can be used to predict and compare reaction fluxes between different experimental scenarios. I will illustrate the advantage of using metabolomics in addition to transcriptomics and proteomics data to test model hypotheses about pathway activity and flux rerouting in algae and higher plants. In addition, I will highlight what data-driven flux-centered analyses imply for the modulation and control of cellular functions in biological systems, as an ultimate goal of systems biology studies.

SP - 6

Solanum Steroidal GlycoAlkaloids: from a Greasy Start to the Bitter End

Aharoni Asaph

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The regulation of metabolic pathways is constantly tuned in order to suit the needs of development and fitness. Our main research objective is to unravel networks of genes and proteins which coordinate the activity of metabolic pathways during plant development and stress response. An integrated investigation of several members of the Solanacea family (particularly tomato, potato and eggplant), rather than studying a single plant, provided us with unprecedented insights to metabolic biology in these species. Most if not all processes characterized, impact to a certain degree key quality, nutritional and post-harvest traits of these crop plants. Integrating cutting-edge transcriptomics and metabolomics tools together with genes co-expression assays were of great value in making several discoveries. In a recent example, combined co-expression analysis and metabolic profiling in tomato and potato led to the discovery of the multi-step, core pathway leading to the formation of the renowned Solanacea glycoalkaloids. This class of cholesterol-derived molecules represent important anti-nutritional compounds in these crop plants. In the presentation, I will highlight several technologies and genetic research tools and the invaluable knowledge on core metabolic pathways obtained through combining them in a single study. Most if not all could be applied in the coming years to the study other pathways of secondary metabolism in model and less studied species of the Solanacea family.

OC - 22

Metabolic genome-wide-association unravels trait architecture and natural variation in tomato primary and secondary metabolisms

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Modern breeding has impacted fruit quality and volatile content, modifying the organolepticquality of tomato crop varieties. To decipher quality trait inheritance and improve it in moderntomato breeding programs, we characterized an association panel including 70 wild relativeaccessions (S. pimpinellifolium), 170 admixed accessions (S.l. cerasiforme) and 50 elitecultivars (S. lycopersicum). GWAS analysis was conducted using 12,000 SNP markers and aset of primary metabolism traits (sugars, organic and amino acids) as well as a broad range ofvolatiles compounds. This study is the first in tomato reporting associations for a large set ofvolatiles at the genome scale. Metabolite content and genetic inheritance varied in a broadrange over different genetic groups. Four genomic regions were detected highlighting clustersof associations for several metabolites. We found significant associations for 84 loci with atotal of 17 traits including glucose, malate or phenylacetaldehyde. Some associations wereconsistent with previously published quantitative trait loci (sugars, guaiacol), while new lociwere identified (phenylacetaldehyde). Local linkage disequilibrium analysis and allele miningallowed the identification of candidate genes to be functionally investigated. These resultsprovide (1) a list of candidate loci and (2) a fast and efficient analytical approach for findinggenetic variants that can be directly used for fruit quality improvement and deciphering thegenetic architecture of complex traits.

OC - 23

Variability in tomato fruit volatiles is interspersed within and across the tomato clade gene pool

José Luis Rambla, Kristty Ortiz, Santiago García-Martinez, Walter Barrantes, Rafael Fernández-Muñoz, Juan J. Ruiz, Antonio J. Monforte and Antonio Granell

Tomato fruits are able to produce a large range of diverse volatile compounds. Some of them play a role in plant communication as they may attract or repel frugivores, and some of these compounds may have been selected during domestication for their contribution to flavour and aroma. The variability in tomato fruit volatiles present in a large representation of the tomato clade pool has been analyzed and revealed some qualitative differences and a huge quantitative variation. The genetic basis of the variability in volatile biosynthesis has also been analyzed in more detail using Recombinant Inbred Lines and Introgression Lines derived from biparental crosses usually involving cultivated and wild accessions. The genetic analysis of such experimental populations enabled the identification of gene regions and markers associated to the accumulation of different volatiles extended over all the 12 tomato chromosomes. Additionally, variation in aroma volatile profiles has been detected as a linkage drag effect in tomato lines which carry different introgression for other traits of interest. This will be exemplified for the case of tomato lines carrying disease resistance genes introduced from wild species.

OC - 24

Evolution of pollination syndromes and scent production in Petunia

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Pollination syndromes, suites of floral traits adopted by different plant species in order to attract specific groups of pollinators, have been deeply studied in the sister species *Petunia axillaris* and *Petunia exserta*. While the hawkmoth-pollinated *P. axillaris* emits high amounts of odor during the night, the hummingbird-pollinated *P. exserta* is completely scentless. QTL analysis revealed two major loci responsible for the production of methylbenzoate, the most abundant and strongest attractive volatile in P. axillaris. One QTL, located on chromosome VII, was assigned to the gene *ODORANT1* (*ODO1*), a well-characterized R2R3-MYB transcription factor involved in scent production. The second QTL was mapped on chromosome II to a specific 1 cM interval with low recombination frequency. Interestingly, this QTL was associated with additional pollination syndrome related traits: visible color, UV absorption, pistil length and stamen length. In order to identify the chromosome II scent gene, we have developed and analyzed introgression lines that harbor the *P. exserta* chromosome II scent QTL in a *P. axillaris* genetic background. High-throughput RNA sequencing conducted on the wild type and introgression lines, together with quantitative PCR expression profiling and phenotypic studies, enable us to perform detailed investigation of differentially expressed candidate genes located within the introgression.

SP - 7

The Tomato Expression Atlas: a new platform for biological discovery with cell-type resolution

Rose, J. K.C.[a], Giovannoni, J.G.[b,c] , Catalá, C.[a,b]., Fei, Z. [b,c], Mueller, L.A.[b], Fernandez, N.[b], Martin, L.B.[a], Nicolas, P.J.[a,b], Snyder, S.I.[a] and Zhang, Y. [b,c]

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Most biochemical and molecular studies involving the extraction of transcripts or proteins from plant organs use a homogenized amalgam of tissues and cell types. This approach limits insights into cell specialization, and lower abundance molecules that are present only in certain cell types are often diluted below the level of detection. There is therefore a critical 'information void' when it comes to annotating and presenting gene expression data. We have been addressing this challenge in the context of understanding the entirety of gene expression during tomato fruit development, by coupling RNA-seq analysis with laser capture microdissection (LCM), which allows the precise isolation of individual fruit cells/tissue types. In addition to resolving gene expression down to the level of cell/ tissue type, this approach has enabled: (i) the identification of previously unannotated genes, demonstrating the value of LCM as a tool for gene discovery; (ii) inferences regarding gene functions, based on the patterns of tissue- or cell type-related expression. We have also been developing computed tomography as a non-invasive imaging tool to create a 3D 'virtual tomato', which includes internal structures, to provide digital a scaffold upon which to present transcriptome, or other 'omics' data sets as a 4D display. All data will be publicly accessible in a new database, the Tomato Expression Atlas. This database includes a novel user interface with a correlation matrix that reveals patterns of co-expressed genes at an unprecedented level of spatiotemporal resolution, thereby optimizing the identification of functionally related suites of genes.

SP - 8

Modelling metabolic fluxes during tomato fruit development

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Our goal is to better understand how metabolism influences cell growth and quality through a systems biology approach centered on fruit development. For that we developed two mathematical models in order to estimate metabolic fluxes during tomato fruit development ; both of them have been parameterized with a large set of samples analyzed for enzyme activities, metabolome and structural traits (Biais et al., 2014). The first model is a compartmented kinetic model enabling the description of sucrose uptake and the partitioning of sugars throughout fruit development (Beauvoit et al., 2014). Interestingly the model pinpointed the strong control exerted by vacuolar carriers on sugar storage and the role of soluble sugars in the vacuole expansion during cell division. The second model is a stoichiometric model describing the central metabolism of heterotrophic plant cells including the balance of cofactors and energy (Colombie et al., 2015). A striking output of this modeling was the occurrence of an excess in energy dissipated just before the onset of ripening supporting the concept of the climacteric crisis. Appearing as an emergent property of the model, this energetic burst has been scrutinized and the amount of starch stored during fruit growth appeared to be crucial.

OC - 25

Systems biology of tomato type VI glandular trichomes: insights into a metabolic cell factory

Gerd Balcke, Nick Bergau, Stefan Bennewitz, Benedikt Athmer and Alain Tissier

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Type VI glandular trichomes are the most abundant type of trichomes on the surface of tomato leaves and stems. In cultivated (*Solanum lycopersicum*) but especially in wild species such as *S. habrochaites*, they provide protection against insects via the metabolites they produce. Despite this important role, little is known about their development and how these metabolic factories are organized. To fill this gap we first established a detailed sequence of developmental stages using a variety of cell biology techniques. This revealed unique features, such as an internal storage cavity, that are adapted to the accumulation and release of large quantities of metabolites. Next we created, screened and mapped a backcross population between *S. habrochaites* and *S. lycopersicum* to identify QTL associated with trichome phenotypes. Several QTL controlling type VI trichome shape could thus be identified. Finally, we performed metabolome, transcriptome and proteome analyses of trichomes and leaves. This multiscale comparative analysis revealed a number of trichome specific features. Our analysis of this dataset focussed so far on the connection between primary and secondary metabolite pathways, revealing distinct strategies adopted by glandular trichomes to ensure an efficient supply of carbon for the production of metabolites. Type VI trichomes depend largely on sucrose imported from the leaf as a carbon source whereas photosynthesis appears to supply energy and reducing power required for metabolic activities but provides little net carbon fixation. This analysis provides a framework to develop a model of these metabolic cell factories.

OC - 26

High resolution characterization of the tomato fruit glycome

Iben Sorensen[b], David Domozych[a], Laetitia Martin[b],

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[b]*Cornell University, USA*

Tomato (Solanum lycopersicum) represents an established model for the study of fleshy fruit development and maturation, and many complex physiological and biochemical processes that determine fruit size, shape and quality traits have been studied in considerable detail. However, homogenized total fruit pericarp tissue has typically been used to study gene expression, or the presence of proteins and metabolites. This approach ignores interior tissues and reveals nothing of the spatial variation in transcript, protein or metabolite accumulation in specific tissues or cell types, resulting in a substantially incomplete picture of fruit development. We are addressing this deficiency by developing the Tomato Expression Atlas: a platform to explore fruit biology at a cell/tissue type specific level of resolution. A component of this study involves an analysis of the fruit glycome, targeting the identity, distribution and dynamics of cell wall polysaccharides. The fruit cell wall undergoes phases of major synthesis, deposition, remodeling and degradation throughout development, but such events, and how they contribute to fruit size, shape and texture, are poorly understood. We are evaluating, at a cell/tissue level of resolution, the expression of a comprehensive catalog of wall related genes through fruit ontogeny.This information will be combined with spatial maps of the glycome, based on a large collection of monoclonal antibodies that recognize defined wall polysaccharide epitopes. This will help resolve the distribution, interactions and dynamic changes in fruit wall composition and architecture, as well as the underlying gene networks, in all fruit tissues throughout development.

Wednesday, October 28th
14:15-14:45

SP - 9

The transcriptional regulatory network underlying fleshy fruit ripening: a case of the interplay between different hormone signally pathways

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The plant hormone ethylene is a major regulator of climacteric fruit ripening but fleshy fruit ripening is more likely controlled by a complex hormonal balance, even though experimental evidences supporting this hypothesis remains elusive. The regulatory mechanisms underlying ethylene action during climacteric fruit ripening are poorly understood, and in particular, the specific role of Ethylene Response Factors (ERFs) in mediating the ripening-associated ethylene responses awaits to be clearly demonstrated. Building on the new tools and genomics resources generated in the tomato, we identified a small subset of *ERF* genes displaying consistent ripening-associated expression pattern and showed that these ripening-related ERFs are connected to the mechanism underlying ethylene- and RIN/NOR-dependent ripening. On the other hand, auxin has been assigned a role in the ripening of fleshy fruits based on the observation that auxin treatment delays ripening. The investigation of the means by which auxin impacts the ripening process in tomato revealed that among all members of the *Auxin Response Factor* (*ARF*) gene family in the tomato, *SlARF2* displays the most remarkable ripening-associated pattern of expression, and that its down-regulation results in strong ripening defects. Hence, SlARF2 emerges as a new component of the regulatory network controlling tomato fruit ripening. SlARF2 is interconnected to known key regulators of fruit ripening, such as *RIN*, *CNR* and *NOR*. Overall, the study provides a new insight into the mechanisms underlying the control of fleshy fruit ripening, and uncovers new avenues towards manipulating the ripening process through means that have not been described so far.

OC - 27

Fruit Ripening Regulation of α-Mannosidase expression by the MADS Box Transcription Factor RIPENING INHIBITOR and Ethylene

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α-Mannosidase (α-Man), a fruit ripening-specific N-glycan processing enzyme, is involved in ripening-associated fruit softening process. However, the regulation of fruit-ripening specific expression of *α-Man* is not well understood. We have identified and functionally characterized the promoter of tomato (*Solanum lycopersicum*) *α-Man* to provide molecular insights into its transcriptional regulation during fruit ripening. Fruit ripening-specific activation of the *α-Man* promoter was revealed by analysing promoter driven expression of *beta-glucuronidase* (*GUS*) reporter in transgenic tomato. We found that RIPENING INHIBITOR (RIN), a MADS box family transcription factor acts as positive transcriptional regulator of *α-Man* during fruit ripening. RIN directly bound to the *α-Man* promoter sequence and promoter activation/α-Man expression was compromised in *rin* mutant fruit. Deletion analysis revealed that a promoter fragment (567 bp upstream of translational start site) that contained three CArG boxes (binding sites for RIN) was sufficient to drive *GUS* expression in fruits. In addition, *α-Man* expression was down-regulated in fruits of *Nr* mutant which is impaired in ethylene perception and promoter activation/*α-Man* expression was induced in wild type following treatment with a precursor of ethylene biosynthesis, 1-*aminocyclopropane*-1-carboxylic acid (ACC). Although, *α-Man* expression was induced in *rin* mutant after ACC treatment, the transcript level was less as compared to ACC-treated wild type. Taken together, these results suggest RIN-mediated direct transcriptional regulation of *α-Man* during fruit ripening and ethylene may acts in RIN-dependent and -independent ways to regulate *α-Man* expression.

OC - 28

Ethylene Response Factors involved in tomato fruit ripening and their connection to master regulators of the ripening process.

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Ethylene Response Factors (ERFs) belong to a large multigene family of transcription factors acting as the main mediators of ethylene-dependent responses by binding to target genes and therefore controlling different aspects of plant development. It is widely accepted that ethylene plays an essential role in fruit ripening, although little is known about the ERF members regulating the ripening process. We present here a comprehensive expression profiling of tomato *ERFs* in wild-type and ripening-impaired tomato mutants (*Nr, rin* and *nor*) identifying the most active *ERF* genes in ethylene- and RIN/NOR-dependent ripening. Out of the 77 ERFs present in the tomato genome, 27 show enhanced expression at the onset of ripening and 28 displayed a ripening-associated decrease in expression, suggesting that different ERFs may have contrasting roles in fruit ripening. Among those exhibiting the most consistent up-regulation during ripening, the expression of 11 *ERFs* is strongly down-regulated in *rin*, *nor* and *Nr* tomato mutants while only 3 are consistently up-regulated. Members of subclass E (*Sl-ERF.E1, Sl-ERF.E2* and *Sl-ERF.E4*) show dramatic down-regulation in the ripening mutants. Further, their expression is highly correlated to other ripening-related genes and connected to the RIN/NOR regulatory network, indicating that these genes might be instrumental to fruit ripening. Overall, the finding provide a new insight for understanding the ethylene-controlled ripening events and show strong evidences supporting the idea that a small subset of ERF genes can be considered as main actors in controlling fruit ripening. Characterization of these transcription factors and their interactions with their target genes is likely to result in a better understanding of the molecular mechanisms of ethylene signaling, underlying ripening in fleshy fruits.

OC - 29

Intronless tandem duplicated class I sHsp genes involved in Solanum lycopersicum (cv Heinz 1706) fruit ripening

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The tomato *S. lycopersicum* (cv Heinz 1706) is a centerpiece of the Solanaceae family and its genome constitutes a reference in the fleshy fruit development study. Therefore, comparing Solanaceae genomic data is possible to detect gene duplication events and hypothesized their mechanisms of origin. In *S. lycopersicum* (cv Heinz 1706) duplication events are the principal source of small heat shock proteins (sHsps) gene family expansion. Goyal (2012) identified an intronless subfamily of cytosolic class I sHsps in the chromosome 6 of in *S. lycopersicum* cv. Ohio 8245. The subfamily it is conformed by three members genes (Solyc06g076540, Solyc06g076560 and Solyc06g076570) alias Sl20.1, Sl117.6 and Sl120.0. The author described 5' UTR stress-responsive elements, probably involved in abiotic and biotic stress response. We found in tomato cv Heinz 1706 a fourth intronless gene member, Solyc06g076520 that conserve a 97.9% nucleotide identity with Solyc06g076560. Its promoter carries similar *cis* stress-responsive elements (ERE,Auxin responsive, GARE, HSE, MYB recognition site and ABRE-like sequence) that may explain fruit developmental and ripening transcript abundance and differential gene expression pattern in RNAseq experiments. A phylogenomic analysis of *Solanum* species suggests that duplication event in the chromosome 6 region occurred before the split of the ancestor of tomato related species and potato (~7.3 mya). Since then, their collinear arrangement of 5 class I *sHsps* genes has been possible maintained by natural selection. Nevertheless, in *S. lycopersicum* cultivars it might have changed due artificial selection.

OC - 30

Detection of QTLs involved in tomato fruit quality in a new genomic library of introgression lines from Solanum pimpinellifolium L

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A new genomic library of introgression lines (ILs) from the Solanum pimpinellifolium L. accession TO-937 was developed in the 'Moneymaker' (MM) genetic background. The process of IL development was accelerated thanks to the implementation of high-throughput single-nucleotide polymorphism (SNP) genotyping during the molecular breeding program. The definitive library consisted of 53 ILs covering 94% of TO- 937 genome. The ILs showed a high level genetic background isogenicity, most of the ILs had no additional introgressions, and in those with such additional introgressions, they were in general very small (< 2 Mb). The genomic IL library was agronomically characterized in three locations (Alginet, Orihuela and Málaga, in the Mediterranean coast of Spain) for several fruit quality traits: fruit morphology, organoleptic characteristics, external and internal fruit color. Genetic and environmental effects varied among traits, being the genetic component more important in fruit morphology traits and soluble solid concentration (SSC) than for fruit acidity and color. Concomitantly, QTL effects for fruit morphology and SSC where more consistent among trials than for fruit acidity and color. A total of 67 QTLs related to fruit quality with consistent effects in at least 2 trials were defined. Additionally, QTLs involved in plant architecture and other vegetative traits were also detected.

OC - 31

A role for the Clp protease complex in chromoplast differentiation and carotenoid biosynthesis during tomato fruit ripening.

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All photosynthetic organisms produce carotenoids, a group of isoprenoid metabolites with industrial and nutritional relevance. Although plants produce carotenoids in all plastid types, they accumulate in largest amounts in specialized plastids named chromoplasts. During tomato (Solanum lycopersicum) fruit ripening, chlorophyll-containing chloroplasts differentiate into chromoplasts that overaccumulate two main carotenoid products, β-carotene (orange) and lycopene (red), causing the fruit color to change from green to orange and finally red when ripe.
Previous evidence indicates that overexpression of genes encoding carotenoid biosynthetic enzymes typically results in modest increases of carotenoid compounds, suggesting that post-transcriptional mechanisms may limit their accumulation. Protein Quality Control (PQC) systems based on chaperones and proteases ensure the correct folding and hence activity of plastidial enzymes. While PQC components regulating tomato carotenoid biosynthetic enzymes remain little known, studies in Arabidopsis thaliana showed that particular families of chaperones and the stromal Clp protease complex directly regulate the activity and accumulation of a number of enzymes involved in carotenoid production in chloroplasts.
Here we explored the potential of manipulating Clp protease activity in tomato fruits to improve their carotenoid profile and hence nutritional quality. Tomato fruits with a reduction in the Clp protease activity were obtained by transient and stable silencing of genes encoding Clp protease subunits. While silencing produced carotenoid-enriched fruits, they stayed orange when ripe. Microscopy examination of transgenic fruit unveiled a strong influence of the Clp protease on tomato chromoplast differentiation. Quantitative proteomic studies are in progress to identify potential targets of this protease during fruit ripening.

OC - 32

Vitamin C and Cell Wall Metabolisms in Tomato: GDP-D-mannose epimerase (GME) a key actor of these interrelated pathways

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The GDP-D-mannose epimerase (GME, EC 5.1.3.18), which converts GDP-D-mannose to GDP-L-galactose, is generally considered as a central enzyme of the major ascorbate biosynthesis pathway in higher plants but experimental evidence for its role in planta is lacking. By using transgenic tomato lines RNAi-silenced for the two GME genes, we could decipher the respective function of GME1 and GME2 proteins during tomato development. Both GME1 and GME2 participate to AsA biosynthesis pathway confirming that GMEs indeed play a key role in the regulation of ascorbate biosynthesis in tomato plants. Regarding the role of the GME activity in the cell wall biogenesis, our data suggest the existence of a specific cell wall-related activity of SlGME1 and SlGME2 according to the considered plant tissue and its developmental stage. On the basis of the gene expression patterns, SlGME2 displayed predominance during the vegetative growth phase, since only RNAi-GME2 transgenic lines exhibited growth delay. On the other hand, GME1 seems to be the major player during the early phase of development of reproductive tomato organs, namely flowers and fruits, as shown by the smaller fruits produced by RNAi-SlGME1 lines. When considered together, these findings confirm the intimate linkage of ascorbate and cell wall biogenesis in tomato plants, and they also reveal the possible existence of specificity of the GME activities related to cell wall that depends on the organ type and its stage of development.

OC - 33

Suppression of ADP-glucose pyrophosphorylase genes affects fruit skin thickness as well as fruit sugar and sugar phosphate contents in tomato (*solanum lycopersicum L.*)

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ADP-glucose pyrophosphorylase (AGPase) is a key regulatory enzyme in starch biosynthesis in plant. In tomato fruit, starch accmulattion at early developing stage is important for sugar content in red-ripe stage. We reported two genes encoding ADP-glucose pyrophosphorylase (AGPase), *AgpS1* and *AgpL1* are involved in the starch accumulation in fruit. However, the physiological function of starch and AGPase have not been well investigated in tomato to date. With this aim, in the present study, we generated RNAi transgenic tomato lines with suppressed expression of the *AgpS1* and *AgpL1* genes, and investigated metabolic alterations in developing fruits. Detailed metabolic characterization in a starch deficient line, *35S::AgpS1*^{RNAi} no. 67, revealed that soluble sugars and glucose-1-phosphate contents were respectively decreased by 19-27% and 19-22% in the transgenic compared to the wild-type at the red-ripe stage. Additionally, fruit malate content increased by about 30% in the RNAi lines compared to the wild-type fruit at immature-green and ripening stages, when the respiratory activity increases. Those results indicate i) that the contribution of starch to the fruit sugar content is about 30%, and ii) that glucan phosphorylase is involved in the starch degradation process, which occurs at early ripening in the fruit. Furthermore, the increase in malate, which was observed in the transgenic fruit suggests that there is a trade-off between starch and malate in developing fruits. Interestingly, the starch deficient lines exhibited reduced fruit skin thickness and hemicellulose content at red ripe stage. Those results indicate multiple roles of starch degradative product in tomato plant.

OC - 34

Environmental Stresses affecting Bemisia tabaci Resistance in Tomato

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Biotic and abiotic stresses are limiting factors in crop production. Plants can be exposed to various stresses concurrently, which may either enhance or decrease the effect of the individual stress. *Bemisia tabaci* is a phloem feeding insect that can affect tomato yield by depleting the plant from photo- assimilates, wilting of leaves, and covering leaves with honey dew on which molts may grow, but the main damage inflicted by the whitefly is caused by the devastating viruses it transmits. Some wild tomato species possess morphological and metabolic adaptations to resist whitefly infestation. Glandular trichomes type I and IV are the first layer of defense, covering the aerial parts of the plants. They have a production, storing and secretory system for specialized secondary metabolites. These trichome types are absent on cultivated tomato, which contain amongst others trichomes of type III and V. These non-glandular trichomes are similar to I and IV except for the glandular head. Trichome type IV production and longevity can be influenced by environmental factors like water and salt stress, light or heat stress and biotic stresses which in turn can affect whitefly resistance levels. We studied the effect of single stresses on glandular trichome type IV and non-glandular trichome type V production as well as whitefly resistance of tomato. A moderate salt stress and six phytohormones were used on *Solanum galapagense* , *Solanum lycopersicum* and an F1 hybrid between these species. In addition, we also studied the effect of ToMV (Tomato Mosaic Virus) infection as a model system for biotic stress. The results will be discussed and show that SA, JA, Cytokinin, Ethylene, Salinity and ToMV affect trichomes density and whitefly resistance in these plants

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SP - 10

Environmental and internal cues that regulate sexual and asexual reproduction in potato

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Potato tuber development is regulated primarily via the photo periodic pathway that also regulates flowering in Arabidopsis and many other flowering plants. While tuberisation is generally induced by the shortening of day length at the onset of Autumn, many Solanaceae including potato and tomato appear to be day-length insensitive for flowering. This day-length insensitivity for sexual reproduction may be seen as consistent with the equatorial origin of potato and tomato. However, it makes the day-length sensitivity of tuberisation all the more intriguing. Some results from the investigation of environmental and internal cues for the regulation of flowering and tuberisation will be presented and discussed.

OC - 35

Gene copy number and allele dosage in autotetraploid potato contribute to gene expression regulation

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Polyploidy is a widespread phenomenon that has great impact on the evolution of plant genomes. The presence of multiple homologs in autopolyploids or homeologs in allopolyploids can afford tolerance to mutation compared to homozygous diploid species, thereby permitting the accumulation of polymorphisms in the form of single nucleotide variation, insertion-deletion events, and copy number variation. At the transcriptional level, gene expression can be affected by dosage and modified expression of homologous gene copies, which can compensate for deleterious alleles or generate novel function through mutation. In this study, we describe the extent of single nucleotide polymorphism, insertions-deletions, and copy number variation on the genome of cultivated potato, Solanum tuberosum, a vegetatively propagated autotetraploid. We find that although dosage of alleles is generally correlated with allelic expression, 7,080 genes (~24% of annotated genes) in RNA-seq analysis of leaf and tuber transcripts from six North American potato cultivars show non-additive allele expression at biallelic loci, suggesting preferential allele expression or degradation. Approximately 25% of genes in each cultivar showed evidence of possible deletions that may be tolerated due to the redundancy of autotetraploid gene copies, although most genes with copy number deletions have significantly lower expression than non-deleted genes in the analyzed leaf and tuber transcripts. The results demonstrate that most genes within autopolyploid genomes are expressed stoichiometrically with respect to their allele and copy number dosages, but that some genes may be subject to regulation that results in non-additive expression.

OC - 36

Phytophthora infestans genetic diversity and effector expression in a single field experiment

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Phytophthora infestans (Mont.) de Bary is the most destructive potato and tomato pathogen worldwide. P. infestans is genetically diverse, fast-evolving and it secretes hundreds of proteins, which promote infection (effectors). These proteins facilitate infection but, when recognized by the host resistance proteins, they trigger defense responses.
We monitored P. infestans population structure and effector expression in the unprotected field experiment in 2014, in Młochów. Half of a leaflet with lesion was frozen in liquid nitrogen and used to analyze expression of effectors: AvrVnt1.1, AvrSmira1, Avr8. The remaining part was used to obtain pure cultures of P. infestans. Total number of tested samples was 89. Mating type, mitochondrial haplotype and diversity of microsatellite markers were determined by PCR. Resistance to metalaxyl was tested on rye A agar media. For virulence scoring, 11 Black`s differentials and new resistance sources were applied in detached leaflets. Expression of effectors was analyzed by a RT-qPCR.
In P. infestans population mating type A2 (73%) and mitochondrial haplotype IIa (75%) were dominating. Most of the isolates were resistant to metalaxyl (55%). We detected 32 unique genotypes among the 89 isolates. 31 isolates were identified as 13_A2 genotype. The mean expression of the AvrVnt1.1 effector was highest among the analyzed effectors and its level was the most variable between tested samples. That is supporting the thesis that P. infestans isolates avoid recognition by the corresponding Rpi-vnt1.1 gene by abolishing the AvrVnt1.1 expression. Expression levels of the tested effectors were related to the SSR genotypes of the isolates.

OC - 37

Use of haploid populations to unravel the heterozygosity of autotetraploid cultivated potato

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The cultivated potato is an autotetraploid, tetratomic, highly heterozygous, vegetative propagated species. Because of their disomic configuration in the same genetic background, haploid populations of potato can be a valuable alternative to study the genetic complexity of quantitative traits. We constructed two gynogenic haploid ($2n = 2x = 24$) populations from cvs. Atlantic (n=135) and Superior (n=58). Fitness and agronomic performance were evaluated in the field in 2014 and 2015 (tuber emergence, plant height, vine vigor, total tuber yield, average tuber weight, number of tubers per plant, specific gravity, and tuber shape). Although tuber dormancy was highly variable, the haploid populations showed extreme phenotypic variation for vigor, tuber number, and total yield. A whole genome re-sequencing strategy has been used to identify genome variants (copy number variation – CNV, single nucleotide polymorphism – SNPs) within the populations. A linkage mapping based analysis will be used to associate sequence variation with phenotypic traits. The role of deleterious mutations as well as allelic dosage in autopolyploid plant vigor can be addressed using the data generated from these populations. These efforts to probe the genetic complexities of potato will serve as a model genomics system for other vegetatively propagated, highly heterozygous crops. (Parallel session - Potato, Oral presentation).

OC - 38

A diploid homozygous self-compatible inbred line in *S. tuberosum*

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In the last century, the genetic research in many crop species has been facilitated by the development of homozygous inbred lines, that allow the generation of dedicated genetic populations. Moreover, populations of recombinant inbred lines (RILs) or backcross inbred lines (Ils) or sets of nearly isogenic lines (NILs) have been developed for advanced quantitative genetics and functional genomics research.
For potato, attempts have been made to induce haploid plants from egg-cells (gynogenesis) or to generate haploid plants from anthers (androgenesis). Though many haploids and double-haploid plants have been generated, these homozygous diploid potato plants were showing severe reduced vigour, which has blocked the use of these genotypes for research. Alternatively, homozygous plants could be generated by selfings. However, diploid potato is self-incompatible and the progress in increasing homozygosity of tetraploid potato is too slow. As a consequence, it has long been perceived impossible to develop homozygous diploid potato lines.
Recently, major breakthroughs have been achieved:

1) The self-incompatibility of diploid potatoes has been overcome by introgressing the *Sli*-gene from *S. chacoense*
2) Inbreeding depression has been overcome by many rounds of crossings, selections and selfings.

The first essentially homozygous self-compatible potato genotypes have already been generated in 2012, but the vigour of these plants was still quite low. The level of homozygosity was assessed by using SNP-markers to investigate the effect of inbreeding on phenotypic traits. A strong correlation between overall level of homozygosity and lack of self-compatibility was observed. By new series of crosses, selections and selfings the agronomic performance of the inbred lines continuously improved.
The development of a fully homozygous self-compatible potato line will be presented. This line is used to introgress resistance genes to *Phytophthora infestans* by marker assisted backcrossing. Moreover, a mutant population is being developed for tilling purposes and to identify useful mutants.

OC - 39

Residual Heterozygosity through cycles of Inbreeding in a Diploid Potato Population

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The majority of commercially cultivated potato (*Solanum tuberosum)* is autotetraploid. The corresponding tetrasomic inheritance pattern inhibits fixation of favorable alleles and linkage groups, complicating potato breeding. Fixation of alleles in diploid potato is expected to be easier, but is hindered by self-incompatibility. We report the generation of self-fertile lines derived from a cross of homozygous DM x heterozygous RH, as well as their maintenance through the S_6 generation. Selections from the F_1, S_3, and S_5 generations were genotyped on the SolCAP Illumina SNP chip platform and the advanced selfed generations exhibited greater than expected heterozygosity. We identified 1,367 heterozygous SNPs in the F_1 progenitor. The S_3 generation was found to have 18% more heterozygous loci than predicted (expected: 171 SNPs), while the S_5 generation ranged from 53-221% more than predicted (expected: 43 SNPs). Some of the retained heterozygosity is possibly caused by artifacts through copy number and/or presence absence variation in the RH haplotypes. In order to ascertain how much of the perceived heterozygosity was valid, we performed whole genome sequencing (WGS) on homozygous androgenic monoploids derived from F_1s of the initial cross. Spuriously heterozygous loci in the monoploid sequencing data were used to filter the SNP chip calls to reveal true retained heterozygosity in the S_3, S_5 and S_6 generations. This research provides insight into the challenges associated with developing a self-fertile diploid potato population.

OC - 40

Small RNA regulation of potato tuber skin and flesh colour

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One of the most eye catching tuber traits of potato is the colour of the skin. Consumers often make purchasing decisions according to the appearance of tubers. Additionally, colour development could be connected to nutritional value as the accumulation of anthocyanins and other metabolites can influence the antioxidant content.
Flavonoids play a major role in tissue colour development and are synthesized through a metabolic pathway that leads to the production of diverse secondary metabolites, including anthocyanins, flavonols, flavones, and pro-anthocyanidins.
Our aim is to investigate the possible role of micro RNAs (miRNA) in tuber skin and flesh colour development. miRNAs are short, single stranded, non-coding RNAs having well established regulatory roles in eukaryotes for a wide range of biological processes. They often target transcription factors which have major roles in many developmental and biochemical traits.
In our study we have found an association between the presence of a potato miRNA (stu-miR828) and purple colour development of tuber tissues. We characterized stu-miR828 which showed differential accumulation levels in white and purple tissues of tuber skin and flesh in several potato cultivars. We have predicted and analysed the target genes of this miRNA and their accumulation in tuber tissues to better understand the regulation of anthocyanin biosynthesis. Additionally, stu-miR828 can give rise to the production of trans-acting siRNAs which might further influence the fine regulation of genes having a role in purple colour development in potato tubers.

OC - 41

Identification of candidate genes associated with quantitative resistance to late blight in *Solanum tuberosum* Group Phureja using association mapping

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Develop potato varieties with resistance to late blight is a permanent task for breeding programs and it constitutes a form to generate crops environmentally friendly. Worldwide most important for potato cultivation is quantitative resistance to *Phytophthora infestans* which causes late blight. Quantitative resistance is an important alternative to resistance mediated by *R* genes, because it confers durable resistance in crops. Association mapping using DNA-based markers is an approach to dissect quantitative traits in plants. We used a candidate gene approach to identify the first genes associated with quantitative resistance to late blight in a diploid collection of 104 *Solanum tuberosum* group Phureja accessions. Twenty-nine candidate genes were selected through an experiment of RNAseq that identified differential SNP allele frequencies for quantitative resistant. Amplicons were generated and sequenced from these genes in the population. Two hundred and thirty nine SNPs were identified and tested for association genetics with resistance to late blight. The phenotypic data were obtained under field conditions by determining the area under disease progress curve in four environments. Five SNPs at two loci were identified associated with quantitative resistance to late blight. One locus *StTinI*, encoded a potato homologue of TMV-induced protein I, and the locus *StTL15A*, encoded a potato homologue of Thylacoid lumen 15 kDa protein. For two loci the minor frequency haplotype was associated with greater resistance. The most resistant individuals in the population were homozygous for the minor frequency haplotype *StTL15A56859831T-StTL15A56859849T*. The *StTL15A* gene presented the highest significance value and effect in the association test.

OC - 42

Diversity of *Fusarium spp.* associated with dry rot of potato tubers in Poland

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Each year over 370 million tons of potatoes are produced worldwide and stored for various lengths of time to provide a continuous supply of this staple food to consumers and industry. During long-term storage, potato tubers are vulnerable to many diseases including potato dry rot, a disease caused by fungi of the genus *Fusarium.*
Species causing potato dry rot vary depending on the region of the world, secondary pathogens and saprophytes may also contaminate potato tubers. In the presented study, samples of potato tubers with dry rot symptoms were collected, and pure fungal cultures were isolated from infected tissues. They were identified as *Fusarium* species using partial nucleotide sequences of the internal transcribed spacer, *translation elongation factor 1-α* and *β-tubulin* genes. Among 149 isolates, 12 species were identified. *F. oxysporum* was the most frequent (45% of the isolates), followed by *F. avenaceum* (12.1%), *F. solani* (10.7%) and *F. sambucinum* (7.4%). Phylogenetic analyses confirmed the species identifications and revealed a high diversity of *F. solani* and a low diversity of *F. oxysporum*. Potential producers of zearalenone and trichothecenes were identified within the obtained isolates using PCR markers. Isolates that were pathogenic to potatoes in laboratory tests were found in four species: *F. sambucinum, F. avenaceum, F. culmorum*, and *F. graminearum*. The effects of increased temperature and mixed inoculum on the pathogenicities of chosen species were evaluated.

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Solanesol: Added value from Solanaceous waste

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Solanesol is a 45-carbon, all-trans-nonaprenol, first isolated from flue-cured tobacco. Solanesol itself has useful medicinal properties and is an intermediate in the semi-synthetic production of coenzyme Q10, a medicine used in the treatment of cardiovascular disease, cancer and atherosclerosis. Levels of solanesol of up to 3% dry weight have been reported to be present in tobacco leaves, the current industrial source [1]. Solanesol is also present in other plants from the Solanaceae including tomato, potato, eggplant and pepper although a detailed survey of levels of solanesol in these species is lacking as is comprehensive understanding of the biosynthetic pathway and its regulation. An alternative economically viable source of solanesol may be developed from the foliage of Solanaceous crops other than tobacco that are normally discarded as waste. For example in potato up to 4 tonnes dry weight of foliage are produced per hectare and this part of the crop is normally destroyed by acid burn down. In this study we have used a genetic approach to investigate the variation in potato leaf solanesol level. QTL analysis of a biparental diploid population has been conducted over two seasons and large effect QTL identified. Over-expression of candidate genes underlying the QTL in a model system adds details to our understanding of the solanesol biosynthetic pathway and its regulation.The research from the DISCO project leading to these results has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement 613513.

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Aims and goals of the Arabica Coffee Genome Consortium (ACGC)

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The genus *Coffea* contains only one tetraploid species: *Coffea arabica*. It is also found that it is the most cultivated coffee species and most popular among consumers. Tetraploidization in plants often generates chromosomal rearrangements that might be quite important. Interestingly, *C. arabica* originated from a cross between two diploid parents, *C. eugenioides* (♀) and *C. canephora* (♂), that gave the allotetraploid in which the two parental genomes are believed to remain separated and not undergo reciprocal recombination. The first goal of the consortium is to obtain a good quality *C. arabica* genome sequence with a clear identification of the two parental subgenomes. This will allow revealing eventual recombination between the two sub genomes and identifying the consequences, at the genome level, of the tetraploidization event. Furthermore, thanks to a wide survey of the genetic diversity of the two parental species, the closest extant relatives have been identified; their genome sequences are also performed in the frame of the consortium. Several additional approaches are also developed, among which the construction of a saturated genetic map in which the two sub genomes are distinguished, an optical mapping of the three sequenced genomes and an IsoSeq sequencing (full length cDNAs). Integration of all the results should allow a clear distinction between the two sub-genomes, which are highly similar. In addition, the re-sequencing of about 30 genotypes covering the genetic diversity of the wild and cultivated forms, will allow confirming if only one tetraploidization event happened in the formation of *C. arabica*. This late part of the consortium project will also allow the identification of a possible neo-diversification induced by man intervention and provide a considerable set of markers and information to breeders for varieties improvement for quality, pest resistance and resilience to climatic changes.

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Improving *coffea* genome assemblies with long read data

Susan Strickler
Arabica Coffee Genome Consortium (ACGC)

Coffea arabica accounts for 70% of world coffee production. It is an allotetraploid (2n=4x=44) with a genome size of approximately 1.3 Gb, derived from the recent (< 0.6 mya) hybridization of two diploid progenitors (2n=2x=22), *C. canephora* (710 Mb) and *C. eugenioides* (670 Mb). In an effort to better understand the complex evolutionary history of *C. arabica*, a dihaploid line has been sequenced, as well as both progenitor species. For *C. arabica*, PacBio sequences were generated at greater than 50x coverage. The present assembly covers 923 Mbp, and has a contig L50 of 178 kbp. The genome assembly has been annotated using RNA-seq, Sanger, 454, and Iso-Seq data. Further assembly refinement is underway to correct collapsed homeologous contigs using SNPs called from Illumina sequence from the closest extant relatives of the parental accessions. Contigs are being anchored using the *C. arabica* and *C. canephora* genetic map. Using a similar strategy, an improved *Coffea canephora* assembly has also been generated. The assembly has a total size of 668 Mbp, a contig L50 of 766 kbp, and has been annotated. *C. eugenoide*s sequencing is currently underway using similar methods. These sequencing projects will produce high quality reference coffee genomes and provide powerful genomic tools to enable more efficient coffee breeding strategies. The results will be made publicly available upon publication at the Sol Genomics Network website (http:// solgenomics.net) and the MoccaDB database (http://moccadb.mpl.ird.fr/).

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Progress report on the sequencing and assembly of the allotetraploid *Coffea arabica* var. Bourbon genome

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It is well known that Coffea arabica is the result of a cross pollination between two Coffea species, very likely Coffea canephora and Coffea eugenioides. The haploid genome size is estimated to be 1.3 Gb and quite evenly distributed between its two sub-genomes. A genome sequencing project is underway to investigate the structure of the allotetraploid genome of arabica. High molecular weight genomic DNA was obtained from entire plantlets of Coffea arabica var. Bourbon and a BAC library was constructed. 175,872 BAC clones were pooled into 96 pools of 384 clones each and the pools underwent DNA sequencing on next generation sequencing Illumina platform. Whole genome shotgun sequencing was also performed on two Illumina libraries with 500 and 800 bp insert size and on one mate-pair library with inserts of two kbp. These libraries were supplemented by the sequencing of cDNA libraries (RNA-seq on Illumina platform) obtained from leaves, root and cherries to use for gene prediction. A preliminary assembly of the genome has been carried out from BAC pools. The assembly is now mapped on the available Coffea canephora1 genome to obtain a consensus and form pseudomolecules. The preliminary bioinformatics analysis of the arabica genome suggests a high degree of polymorphism between its sub-genomes, in line with the allotetraploid constitution of the Coffea arabica genome.

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Coffea, *Rhazya*, and the evolution of the Gentianales

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Lacking genomic data from other plants in Rubiceae, the chromosome-level evolution of the coffee genome over the last 60 or 70 million years can now be studied via comparisons with recently sequenced genomes in the sister family Apocynaceae. We compared a high quality assembly of Rhazya stricta to the Coffea canephora1 and the Vitis vinifera2 genomes to infer major evolutionary events leading from the core eudicot ancestor to a putative Gentianales ancestor, and thence to its modern asterid descendants. The strategy was to trace the fates of the 21 ancestral chromosomes emerging from the gamma hexaploidization at the root of the core eudicots, as readily reconstructed from the Vitis genome, an approach that controls for paralogy/orthology ambiguities and extensive fractionation3. We identified rearrangements leading from the ancestral core eudicot to the most recent common ancestor of Rhazya and Coffea and rearrangements leading from this common ancestor to each of Rhazya and Coffea, by locating rearrangement breakpoints within eight of the largest Rhazya superscaffolds and the corresponding Coffea chromosomes, accounting for most of the major gene order changes in the extant genomes. We find only a limited amount of rearrangement between the core eudicot ancestor and the Gentianales ancestor, or between the latter and Coffea, but much more in the lineage leading to Rhazya.

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Integration of C. *canephora* into Arabica variety Catimor: a genomic view

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Arabica coffee has long been recognized for its high cup quality and accounts for 75 - 80% of the world's coffee production. A high-density genetic map of Arabica coffee was constructed using an F2 population derived from Tall Mokka plant (MA2-7) x Catimor plant (T5175-1). Tall Mokka and Catimor have distinctively different cupping quality and leaf, cherry, and bean morphology. A linkage map of Arabica coffee was constructed using 61 F2 individuals and 1,361 AFLP markers. A total of 1,088 markers were mapped on 42 linkage groups. The total length of the linkage map is 689 cM with a marker density at 1.58 markers/cM. Among the 1,088 mapped markers, 612 are Catimor-dominant (56%) and 476 are Mokka-dominant (44%). The resulting map consists of 21 linkage groups with purely Catimor-dominant markers and 21 linkage groups with Mokka-dominant markers, indicating that there is no meiotic recombination between parental chromosomes. The recombination appeared to have occurred between homeologous chromosomes within each parent with reduced rate at about 20% of that between homologous chromosomes. Catimor was derived from a cross between C. arabica coffee variety Catuai and a C. canephora variety with doubled chromosome number and then backcross to Catuai. The integration of C. canephora genomic fragments with rust resistance is likely the cause for lack of chromosomal recombination between Tall Mokka and Catimor. The genomes of these two parents and 70 F1 individuals were sequenced, and the integration of C. canephora genomic fragments into Catimor genome is being explored.

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Genome Wide Association study for drought tolerance and other agronomic traits of a *Coffea canephora* population

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Recent technological advancements and cost decreases on DNA-sequencing technologies allowed the completion of a reference sequence of the *C. canephora* genome. In due time, perhaps with some delay, in view of the economic and social importance of coffee worldwide as well as its perennial aspect (*vis à vis* annual crops), to provide the research power to face the challenges lying ahead, imposed by the real/potential climate changes impacts. Studies on a genome-wide scale are now being performed allowing researchers to narrow down some key molecular players that will certainly be applicable to fast and cost-effective molecular breeding programs. This work describes a Genome Wide Association Study (GWAS) for drought tolerance and other important agronomic traits such as yield of a *C. canephora* conilon population, cultivated in Planaltina-DF (1175m altitude) at the experimental field of Embrapa Cerrados. Phenotyping started in 2012, evaluating characteristics such as vigor, secondary branching, leaf-rust susceptibility, precocity and fruit load. Furthermore, the yield of each plant was measured for three consecutive years (2012-2014) and the predawn-leaf water potential (Ψam) of 400 plants was also evaluated under field conditions (drought season of 2012/2013). Genotyping was performed using the nextRAD technique provided by SNPSaurus (http://snpsaurus.com/), yielding 11.230 SNPs with a call rate above 80%. Population structure was determined using the admixture model of the software STRUCURE. Marker-trait associations (MTAs) studies were conducted employing mixed linear model (MLM) analysis with optimum compression and kinship matrix (TASSEL). Significant MTAs were found and will be presented.

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Identification of aquaporins in *Coffea arabica* L., gene expression study and correlations with plant water relations and hydraulics

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Plant aquaporins (AQPs) belong to a large superfamily of conserved membrane proteins called major intrinsic protein (MIPs), involved in the membrane transport of water and other small solutes. Aquaporins are known to play major roles in the regulation of plant water balance and transport, as well as in growth regulation and response to abiotic stress factors. Our research identified candidate coffee AQP genes by screening a proprietary C. arabica transcriptome database, resulting in the selection of eight putative aquaporins. A phylogenetic analysis was performed using previously characterized MIPs from Arabidopsis thaliana and Solanum tuberosum, specifically assigning the coffee sequences to the Tonoplast (TIP) and Plasma membrane (PIP) MIP's subfamilies.
After bioinformatic analysis, the possible functional role of putative Arabica AQPs was explored by means of physiological and biomolecular experiments. Hydraulic conductance and aquaporin gene expression were analyzed on leaf and root tissues of two-year-old plants (C. arabica cv. Pacamara), under two different experimental conditions.
The first experiment tested the plants before dawn and at mid-day, to verify the influence of light and photosynthesis on AQP activity. In a second experiment, we compared plant hydraulic response to different water stress level as eventually affected by changes in aquaporin expression levels.
The results shed the first light on the possible roles of aquaporins in the regulation of C. arabica water balance, opening a new field of research that could become increasingly important for the sustainability of coffee cultivation in a scenario of global climate change.

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Diterpenes metabolism and related genes in coffee

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Cafestol and kahweol are two diterpenes specific to coffee genus. They are pentacyclic alcohols from the kaurane family, mainly found esterified with various fatty acids. Despite they have been receiving more and more attention in recent years due to their different physiological effects, little is known about their synthesis. However, the actions they have been attributed and their substantial amount in the lipid fraction of the coffee bean make it important to discover their biosynthesis pathway. Based on an intensive bibliography and current state of the art review, the aim of the present work was first to establish a draft for diterpenes metabolism in coffee, focusing on the diterpene pathways and genes already known from model plants such as Arabidopsis, tobacco and maize. According to the metabolic pathway proposed here and the enzymes expected to be involved, the recently published *Coffea canephora* DH200 genome was used for genome-wide candidate genes identification using protein sequences from model plants as queries. This search uncovered 70 genes, including partial and complete sequences, belonging to diTPS families including CPS and KS-type enzymes, CYP450 superfamily including KO and KOA-type enzymes, and transcription factors. A similar approach was performed using the *Coffea arabica* genome that is currently under assembly, and found 100 genes. Then, to assess specialized diterpenes metabolism during coffee bean maturation, cafestol and kahweol were quantified. In parallel, transcriptome analyses were performed to identify those genes that are transcriptionnaly active/specifically involved in their synthesis.

SP - 12

Improvement of pepper fruit quality by molecular breeding

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For improvement of pepper fruit quality, identification of major genes controlling quality traits and developing molecular markers for use in marker-assisted selection, we conducted several quantitative trait loci (QTL) studies in populations segregating for various quality parameters. These include fruit size and shape, color, pungency, total soluble solids, flavonoid content and water loss during post- harvest storage. For post-harvest water loss we identified two linked QTLs in chromosome 10. Near-isogenic lines (NILs) differing for the QTLs indicated that post-harvest water loss is partly controlled prior to fruit ripening. Furthermore, reduced post-harvest water loss was associated with delayed over-ripening of the fruit on the vine. For flavonoid content, we constructed a highly saturated map of the segregating population using the genotyping by sequencing (GBS) approach. We were able to identify several major effect QTLs controlling multiple metabolites and candidate genes underlying these QTLs. For chlorophyll content in the immature fruit, we identified two major QTLs in chromosomes 10 and 1. The transcription factor CaGLK2 was identified as the likely candidate for underlying the QTL in chromosome 10. We are currently conducting high-resolution mapping at the QTL region in chromosome 1. All together, we were able to identify valuable genetic resources, major QTLs and molecular markers that will be implemented in breeding programs towards improved fruit quality.

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A high quality eggplant (Solanum melongena L.) genome draft allows the mapping of phenotypic and metabolic QTLs

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Eggplant (Solanum melongena L. 2n = 2x = 24, projected genome size 1.1 Gbp) belongs to the Solanaceae family, which also includes a number of other important crops like tomato, potato, pepper and tobacco, whose genome sequences are available. Both its membership to the subgenus Leptostemonum and origin from Asia make eggplant of particular interest for comparative genomic analyses within the Solanaceae family.
A high quality eggplant genome assembly was produced from the inbred eggplant line '67/3', which is the male parent of a mapping population composed of 167 F6 RILs, using both Illumina sequencing and Bionano Genomics optical mapping. The hybrid assembly covered 1.2 Gb, with an L50 of >3 Mb. Of these, over 900 Mb were covered by Illumina contigs and anchored to the genetic map obtained with the RIL population. RNA-Seq assisted annotation using Maker resulted in about 39k protein-coding genes. The genome assembly, annotation and RNA-Seq data are being used for comparative analyses with other Solanaceae genomes.
The RIL population has been extensively phenotyped for both visual and metabolic traits. Examples of preliminary QTL mapping for several of these traits will be presented.

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MicroRNA156/7-mediated control of anthocyanin pigment accumulation in eggplant fruit peel

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MicroRNAs (miRNAs) are known to play key roles in many developmental processes and stand at the core of regulatory networks. MiRNAs have been sequenced and studied in some aspects of Solanaceae growth and development, but very little is known regarding their role in fleshy fruit.
We have taken miRNA156/7 as an example of a well-conserved plant miRNA, this miRNA is known to directly target a family of Squamosa Promoter binding-Like (SPL) transcription factors through sequence-specific recognition, and has been shown to play a profound effect on the timing of vegetative phase change and plant architecture in a wide variety of plant species.
We have shown that overexpression of miRNA156/7 in eggplant has a distinct negative effect on the production of anthocyanin pigments at the early stages of fruit development, whilst not influencing the accumulation of the yellow pigment naringenin chalcone at later stages of ripening. The point of action of miRNA156/7 within the phenylpropanoid pathway is therefore very specific. In order to elucidate this, we have performed detailed transcriptomic and metabolomic analyses at several stages of ripening in miRNA156/7 overexpression lines. This has enabled us to identify several direct SPL gene targets of the miRNA, plus putative downstream targets of the SPLs which may represent the point of regulation within the phenylpropanoid pathway. Validation of these targets is currently ongoing. The study of eggplant fruit displaying a unique pattern of flavonoid pigment accumulation in the peel has provided us with insights of how a well conserved miRNA controls metabolic processes in a species-specific manner.

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Toward a characterization of the pepper host resistance effect on the gene expression of the pathogenic *Phytophthora capsici*

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The Oomycete *Phytophthora capsici* is a polyphagic pathogen which attacks various plant species of agronomic interest. It is notably known to cause important damages on pepper. A few partially resistant pepper genitors have been described and to identify the effect of the pepper resistance on the gene expression of *P. capsici*, a RNA-Seq analysis was used. Two pepper accessions (one resistant and one susceptible) were inoculated with two *P. capsici* isolates separately. Infected plant tissues were collected at two times after inoculation in three replicates giving 24 biological samples. Samples' total RNAs were extracted and sequenced by the Illumina technology. 37 million paired-end reads were analyzed on average per sample. Between 73.8 and 80.1 % of them mapped to the released *P. capsici* and pepper genomes, with 0.004 to 6.010 % of the mapped paired reads to the reference *P. capsici* gene models and 93.9 to 99.9 % to the pepper transcriptome contigs. Focusing on the pathogen, we observed 5528 genes expressed among the 20 296 *P. capsici* gene models. Comparison analysis between samples highlighted 294 genes with significant differential expression pattern according to the resistance level of the pepper genitors. A preliminary genomic study revealed that some of those genes are involved in pathogenicity. The completion of this study should deliver new tools to aide development of genetic resistance in pepper. This project was supported by Agropolis Fondation under the reference ID « Protéines pathogènes » 1300-002, and by INRA-DBAP under the reference ID « EffeCaps ».

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Development and characterization of a 19K Illumina Infinium genotyping array for pepper (*Capsicum*)

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Based on genome re-sequencing data, a genotyping array has been developed for the genetic analysis of pepper (Capsicum). For this, 12469 SNP markers were selected that were polymorphic both in hot and blocky type peppers. Furthermore, 2909 additional markers with polymorphism in blocky types and 3622 markers with polymorphism in hot types exclusively, were added resulting in a total of 19000 markers. After array manufacturing, 16405 markers (86.3%) were defined as functional in terms of genotyping assays. Analysis of these markers with a set of pepper inbred lines and hybrids revealed 14877 (90.7%) of the functional markers that could be scored in a wide range of pepper lines and hybrids. 13760 markers displayed an allele frequency of more than 1% (9921 had an allele frequency of more than 10%). Through the analysis of a genetic mapping population, 5654 markers could be genetically mapped on the 12 chromosomes, producing a high-quality genetic map. The results show that this array provides a valuable resource for genetic analyses in pepper, including genetic fine-mapping, genome-wide population analysis, association mapping, and the identification of introgressed segments from wild species into cultivated pepper.

SP - 15

Multiple de novo genome sequences of hot pepper provide insights into species diversification in *Capsicum spp*.

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A diversity of Capsicum species has been derived from extensive genomic and phenotypic variations. Here, we report high-quality de novo genome sequences of two Capsicum species (C. chinense and C. baccatum) as multiple reference genomes of hot pepper. A total of 95 % of C. chinense (3.07 Gb of 3.21 Gb) and 76 % (3.22 Gb of 4.2 Gb) of C. baccatum genomes were assembled. Of these genome assemblies, 87.3 % (2.63 Gb) and 87.2 % (2.79 Gb) were anchored to 12 chromosome pseudomolecules, respectively. Comparative analysis with the existing pepper reference genome (C. annuum) revealed that the amount of ribosomal DNA sequences in C. baccatum was more than 10 fold compared to the other pepper genomes. Athila, a subgroup of Gypsy family, was excessively accumulated in C. baccatum and increased the genome size in this species compared to the others. Phylogenetic analysis revealed that rhw speciation of C. baccatum and C. chinense has occurred at 1.7 and 1.1 million years ago, respectively. We identified correlation of proliferation of LTR retrotransposons and acceleration of gene expression change, gene duplication, and large genomic variations following the speciation of the pepper plants. Our results support that rapid accumulation of LTR-retrotransposons during the speciation time has contributed diversification of the three pepper genomes. The multiple pepper genomes will serve as important resources for comparative and population genomics as well as evolutionary studies of the genus Capsicum.

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A strategy for broadening the genetic base of eggplant using wild relatives as donors of variation

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Like many domesticates, common eggplant (Solanum melongena) has a narrow genetic base when compared with its wild relatives. Unlike other important vegetable crops, up to now few efforts have been made to use wild species for developing new eggplant varieties. We have initiated a project funded by Global Crop Diversity Trust aimed at using a comprehensive and systematic strategy to broaden the genetic base of eggplant, in particular for adaptation to climate change, by exploiting genetic resources of its wild relatives. We are in the process of developing a set of introgression lines based on backcross generations to eggplant of a hybrid between S. melongena and the drought tolerant wild species S. incanum. Several types of molecular markers, including SSRs and SNPs have been used in the backcross process. Solanum incanum introgression lines are being crossed to local varieties from Southeast Asia and West Africa, areas highly vulnerable to climate change, in order to develop drought tolerant materials. Also, eggplant interspecific hybridization has been used to obtain interspecific hybrids with 9 wild species of the primary and secondary genepools. In addition, by using embryo rescue techniques, interspecific hybrids have been obtained with tertiary genepool species S. torvum. New crosses recently performed have also allowed recovering putative hybrids with three other species of the secondary genepool and with tertiary genepool species S. elaeagnifolium, which is highly tolerant to drought. Interspecific hybrids are being backcrossed to the cultivated S. melongena and successful backcrosses have been obtained up to now for six wild species. Screening for drought will be performed in these materials. In addition, new sets of introgression lines will be obtained. The strategy we are using will allow broadening the genetic base of eggplant, which will ultimately result in new varieties with greater resilience to stresses resulting from climate change.

OC - 56

De novo transcriptome sequencing in four non-model species in genus *Solanum* (*S. incanum, S. aethiopicum, S. muricatum* and *S. caripense*): Analysis and molecular markers detection for breeding purposes

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Transcriptome sequencing has become an essential tool to generate genomic data in species where genomic resources are sparse. Here we present the results of transcriptome sequencing of four Solanum species (S. incanum, S. aethiopicum, S. muricatum and S. caripense) using Illumina HiSeq 2000 platform, the comprehensive analysis of their de novo assembly and molecular marker discovery. S. incanum is considered as the wild ancestor of common eggplant (S. melongena L.), exhibiting a great amount of phenolics and tolerance at drought. Scarlet eggplant (S. aethiopicum) is a cultivated African eggplant, showing resistance at some biotic stresses and is used also as rootstock for common eggplant (S. melongena). Pepino (S. muricatum) is a neglected herbaceous domesticate native to the Andean region grown for its juicy fruit and is phylogenetically close to potato and tomato. Solanum caripense is a wild relative of pepino showing high levels of soluble solid content and phenolic acids and resistance to Tomato Mosaic Virus and to Phytophthora infestans. More than 100 million raw reads were obtained for all species which were assembled with Trinity software. We obtained 83,905 unigenes for S. incanum, 87,084 for S. aethiopicum and 75,832 for S. muricatum. As S. caripense is phylogenetically very close to pepino we mapped its reads against the pepino assembled transcriptome instead of assembling them separately. All unigenes were functionally and structurally annotated to identify potentially encoding proteins and orthologs, to assign GO terms, EC number and KEGG pathway, and to predict ORFs and introns. More than 1000 EST-SSRs were discovered in each transcriptome as well as tens of thousands of intraspecific and interspecific SNVs (SNPs and INDELs). The genomic information and markers generated in this study will be extremely useful in the breeding programs of eggplant and pepino, as well as for tomato and potato and to perform marker-trait association and QTL analysis studies.

SP - 13

Small genetic changes, big phenotypic effects: the evolution of trichome specialized metabolism in *Solanum* and beyond

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Throughout human history plant-derived natural products were used in medicines, in cooking, as pest control agents, and in culturally important rituals. Plants produce these rapidly diversifying specialized metabolites as protective agents and to mediate interactions with beneficial organisms. Plant glandular secreting trichomes are epidermal protuberances that produce structurally diverse specialized metabolites, including acylated sugars. *In vitro* reconstruction of the cultivated tomato insect protective acylsucrose biosynthetic network showed that four BAHD acylsucrose acyltransferase enzymes are sufficient to produce the full set of naturally occurring compounds. Comparative biochemistry with the *in vitro* reconstitution system identified simple changes in enzyme structure leading to the acylsucrose diversity in trichomes of wild tomato. Ongoing work on trichome metabolites across the Solanaceae led to the identification of diverse acylated compounds and BAHD acyltransferases involved in the synthesis of novel compounds in *S. quitoense* (Naranjilla) and the basal species *Salpiglossis sinuata*. The talk will emphasize the power of analyzing biochemical processes through integration of comparative genomics with metabolite profiling, homology modeling, *in vitro* biochemistry and reverse genetics.

OC - 57

Using a backcross population to investigate the morphology and metabolism in type VI glandular trichomes in tomato

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Glandular trichomes are specialized epidermal cells and important "biofactories" of secondary metabolites such as terpenoids necessary for the defence of plants against biotic stress. Type VI trichomes represent the major type of glandular trichomes in tomato and consist of a single stalk cell attached to a four-celled head via an intermediate cell. There are clear morphological differences between these trichomes in *Solanum habrochaites* compared to all other tomato species. While in the former the head cells are arranged around a large central cavity forming a ball like structure, the shape in the others such as *Solanum lycopersicum* resembles a four-leaved clover and the central cavity is much smaller. The cavity is the place where the secondary metabolites are stored. Cultivated tomatoes accumulate far lower amounts of secondary metabolites in their type VI trichomes, leading them to be more susceptible to pests. Here, we present a backcross population of *S. habrochaites* LA1777 and *S. lycopersicum* var. *cerasiformae* WVa106 that we used to identify QTLs contributing to the trichome shape. In addition we could use this population to identify genes necessary to produce *α*-santalenoic and *endo-β*-bergamotenoic acid, the main secondary metabolites in the type VI trichomes in LA1777. We show that in addition to the previously characterized *Z,Z*-farnesyl diphosphate synthase (zFPS) and santalene and bergamotene synthase (ShSBS), three additional genes are required to produce these sesquiterpene carboxylic acids. Progress in the characterization of these genes will be presented.

OC - 58

Concerted transcriptional-metabolic remodeling underlies the transition from green-fruited to red-fruited tomato species

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Cultivated tomato (*S. lycopersicum*) is part of the lycopersicon section, comprising 13 species. In the majority of species fruits stay green throughout ripening, while in a few species (*S. cheesmaniae*, *S. galapagense*, *S. pimpinellifolium* and *S. lycopersicum*) they show a change in color from green to yellow/orange or red. In order to understand the molecular basis of these differences in ripe fruit color, we conducted combined metabolic and transcriptional profiling of two green-fruited (*S. neorickii* and *S. arcanum*) one yellow-fruited (*S. cheesmaniae*) and two red-fruited (*S. pimpinellifolium* and *S. lycopersicum*) species. Pigment composition of leaves and fruits at different stages of development was analyzed by HPLC-PDA, and gene expression was analyzed using RNA-Seq. The results suggest that a complex remodeling of multiple structural and regulatory genes involved in carotenoid and chlorophyll biosynthesis, as well as of genes involved in the general regulation of fruit ripening and of ethylene biosynthesis/perception underlies the differences between green- and red-fruited tomato species.

OC - 59

The AP2/ERF-type Transcription Factor GLYCOALKALOID METABOLISM 9 Regulates Cholesterol and Steroidal Alkaloid Biosynthesis in the Solanaceae

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Steroidal alkaloids (SAs) are plant defense compounds produced in the *Solanaceae* family and considered as anti-nutritional factors in the human diet. Recently, a multi-step pathway for SA biosynthesis was proposed starting from cholesterol up to the glycosylated SAs (i.e. steroidal glycoalkaloids; SGAs). Here, we discovered that GLYCOALKALOID METABOLISM 9 (GAME9), an APETALA2/Ethylene Response Factor (AP2/ERF), regulates SGA biosynthesis. GAME9 is closely related to transcription factors regulating the biosynthesis of nicotine (a pyridine alkaloid) in *Nicotiana tabacum* and terpenoid indole alkaloids in *Catharanthus roseus*. Downregulation of *GAME9* in tomato resulted in a considerable reduction of the main SGA α-tomatine levels in leaves. Conversely, overexpression of *GAME9* caused an increase in α-tomatine in tomato and α-chaconine and α-solanine in potato, together with an altered sterol composition. Altered *GAME9* expression affected genes involved in the biosynthesis of SGAs and the upstream cholesterol precursor pathway. Some but not all of these genes are direct targets of GAME9. The results pointed to additional transcriptional regulator(s), acting either downstream or interacting with GAME9, that might be taking part in SA control. Indeed, in recent work, we were able to identify such factor, a GAME9-associated regulator that is likely involved in the control of GAME9 targets. Hence, these findings provide insights to the transcriptional regulation of SA biosynthesis and means for manipulation of these potent metabolites in *Solanaceae* crops in which high SGA levels affect product quality.

OC - 60

Reduced Steroidal Glycoalkaloid Levels Affects *Solanum tuberosum* Biotic Stress Resistance

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Steroidal glycoalkaloids (SGAs) are toxic to humans at high levels, but their role in plant protection against pests and pathogens is less clear and is investigated in current study. Biosynthesis of SGA starts with cholesterol, which undergoes a series of enzymatic reactions to produce SGA backbones. Sequential glycosylation of the nitrogenous aglycone leads to the accumulation of diverse SGAs in plants. Solanine and chaconine are the major SGAs in cultivated potato, *S. tuberosum*. Levels of SGAs can be manipulated by modifying genes encoding enzymes in the SGA biosynthesis pathway. In this study, *Glycoalkaloid Metabolism 4 (GAME4)*, a gene encoding a cytochrome P450 enzyme involved in an oxidation step in the conversion of cholesterol to SGAs, was knocked down in *S. tuberosum* cv. Bintje using RNA interference (RNAi). The levels of solanine and chaconine in potato leaves were reduced by 2-10 times in the *GAME4* knockdown lines compared to wild type. Untargeted metabolite profiling of the foliage extracts from wild type and *GAME*-silenced plants was performed by using LC-MC qTOF. The results revealed that the reduction of SGA levels in *GAME4* RNAi lines was accompanied by increase in other metabolites, such as saponins, phenylpropanoids and methyl jasmonates. Biotic resistance of *S. tuberosum* against *Phytopthora infestans* and *Verticillium dahliae* was examined under the altered metabolic profile. Pathogen infection studies showed that the *GAME4*-silenced plants had increased resistance against *Phytophthora*, whereas they were more susceptible to *Verticillium*. The *GAME4* RNAi plants, however, did not affect Colorado potato beetle (CPB) larval growth and development.

OC - 61

Tomato, a cell factory for the production of high value ketocarotenoids

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Carotenoids are natural colorants with potent antioxidant properties. Animals and humans acquire carotenoids through their diet, primarily from crop plants (roots, leaves, shoots, seeds, fruit and flowers). Ripe tomato fruits contain high basal levels of carotenoids in specialised organelles called chromoplasts. The pigments traditionally associated with tomato fruit are lycopene and β-carotene (provitamin A). Ketocarotenoids, such as astaxanthin, are high value pigments typically produced by chemical synthesise since biological sources are rare with a few algae and other microorganisms capable of producing these compounds. To create a high ketocarotenoid containing tomato fruit, the carotene ketolase (*Crt*W) and hydroxylase (*Crt*Z) have been introduced into a high β-carotene tomato line. High level of ketocarotenoid production was achieved in ripe tomato fruit. The utility of the germplasm has been demonstrated through scaled up in order to evaluate their potential as feed additives in aquaculture. A feed trial was carried out using freeze-dried tomato powder as colorant in the fish feed and compared to commercial feed containing synthetic astaxanthin. Results show that tomato-derived ketocarotenoids can colour trout to the same level as synthetic astaxanthin. Presently, the chemically synthesised product represents 20% of the total feed costs. The present study demonstrates the generic used of tomato fruit as a cell factory to deliver a renewable source of high value compounds.

OC - 62

An O-methyltransferase modifies accumulation of methylated anthocyanins in seedlings of tomato

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Anthocyanins contribute to the appearance of fruit by conferring to them a red, blue or purple colour. In a food context they have also been suggested to promote consumer health. In purple tomato tissues, such as hypocotyls, stems and purple fruits, different anthocyanins can accumulate. These molecules have characteristic patterns of modification including hydroxylations, methylations, glycosylations and acylations. The genetic basis for many of these modifications has not been fully elucidated, nor has their role in the functioning of anthocyanins been established.

In this work, AnthOMT, an *O*-methyltransferase (OMT) mediating the methylation of anthocyanins has been identified and functionally characterized using a combined metabolomics and transcriptomics approach. Gene candidates were selected from the draft tomato genome and subsequently, their expression was monitored in a tomato seedling system comprising three different tissues and involving several time-points. In addition, we also followed gene expression in WT red and purple transgenic *Ros/Del* tomato fruits. Of the 57 candidates identified, only a single OMT gene showed patterns strongly correlating with both the accumulation of anthocyanins and expression of anthocyanin biosynthesis genes. This candidate (*AnthOMT*) was compared to a closely related Caffeoyl-CoA OMT by recombinant expression in *E. coli* and then tested for substrate specificity. AnthOMT showed a strong affinity for glycosylated anthocyanins, while other flavonoid glycosides and aglycones were much less preferred. Gene silencing experiments with *AnthOMT* resulted in reduced levels of the predominant methylated anthocyanins. This confirms the role of this enzyme in the diversification of tomato anthocyanins.

OC - 63

Carotenoid biosynthesis and accumulation in fleshy fruits of *Lycium* L.

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Lycium L. is a genus of Solanaceae containing about 80 species distributed in the temperate and subtropical zones. *Lycium* species are mostly found in dry, semi-saline environments. Chinese Pharmacopoeia (2010) recorded Lycii Fructus (*Gouqizi*, dry red fruit [RF] of *L. barbarum*), and Lycii Cortex (*Digupi*, dry root bark of *L. chinense* and *L. barbarum*). Black fruits (BF) of *L. ruthenicum* have been used as folk medicine, especially in Tibetan and Mongolian medicine. Therefore, wolfberry (or Goji, Gouqi) nowadays in China refers to the products prepared from *L. chinense*, *L. barbarum*, and *L. ruthenicum*, which is one of the most famous anti-aging herbs. Transcriptome sequencing and chloroplast development have been investigated for identification of key genes involved in the biosynthesis and accumulation of carotenoids, especially zeaxanthin dipalmitate. The failure of the chromoplast development in BF causes low carotenoid biosynthesis levels and continuous carotenoid degradation, which ultimately leads to undetectable carotenoid levels in ripe BF. In contrast, the successful chromoplast biogenesis in RF furnishes the sink necessary for carotenoid storage. The abundant zeaxanthin accumulation in RF is primarily determined via the high levels of carotenoid biosynthesis, transportation, and storage, as well as the lack of carotenoid degradation, which are regulated at the transcriptional level. Function of several key genes will be characterized and reported.

OC - 64

Diversity in fruit color in the tomato clade-do we have the answers?

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Tomato clade has *S. lycopersicum* as sole domesticated species and several wild relatives differing greatly in their morphology as well as the ecological niches they inhabit. The wide variation in the morphology is also extended to their fruit coloration, as wild species are predominantly green fruited except one red fruited and one orange fruited species. However, the mechanisms governing inter-species diversity in fruit coloration in the tomato clade are largely unknown. We used a proteomics approach to decipher the molecular basis of diversity in carotenogenesis by using *S. habrochaites* (green fruited, SH)*, S. pimpinellifolium* (red fruited, SP), *S. galapagense* (orange fruited, SG) and *S. lycopersicum* (cultivated tomato, SL). Our analysis revealed that the β-branch of the carotenoid biosynthetic pathway was favored during the transition from green to red fruited species. Moreover, higher ethylene emissions in wild species than SL indicated an attenuation of ethylene formation in cultivated tomato. Comparison of the proteomes of above wild species with SL revealed large number of differentially expressed proteins in wild species at all developmental stages. Consistent with the green fruited nature and chlorophyll retention in SH fruits, the functional class PS-light reaction was upregulated and SH fruits retained PSII activity. Chlorophyll degradation was also compromised in SH fruits as they accumulated 10.5 fold higher Pheophorbide a levels than SL. Targeted peptide monitoring revealed variable abundance of carotenogenic proteins in the fruits of wild species compared to tomato. Our results indicate that the carotenoid biosynthetic pathway, which is conserved across higher plants, is subject to species-centric regulation and the data would be presented.

SP - 14

Photosynthesis as a target for improvement – can it be done and would it be useful?

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Photosynthetically active radiation is, via photosynthesis, the driving force for plant growth and the relationship between absorbed irradiance (or PAR) and crop growth is surprisingly simple. Crop growth is often carbon limited (ie growth could be improved by increased assimilation). Simple solutions for improving crop yield are becoming exhausted and consequently future large increases in yield are likely to require an improvement of photosynthesis at the leaf and canopy level. There are two main strategies for doing this: one is fundamentally based on a genetic engineering approach to fix those parts of photosynthesis that are identified as deficient (such as rubisco), the other is based upon exploiting natural variation for photosynthesis and breeding for better photosynthesis. These two options are, of course, not mutually exclusive. I will focus on leaf level photosynthesis and the exploitation of natural variation.
Photosynthesis is a complex trait both genetically and physiologically, and we basically have only a limited understanding of the genetics of photosynthesis. Using Arabidopsis as a model (but also Brassica and soon Solanum) and chlorophyll fluorescence based phenotyping we have begun to analyse the genetics of photosynthesis. The data I will present are derived from measurements of the responses of Arabidopsis to step changes in irradiance, but it would be possible to tell a similar story for the data obtained from Brassica. In fact provided it is possible to phenotype the plants and the appropriate genetic tools are available there seems no reason why our experience could not be extended to other species.

OC - 65

Light signaling pathways are recruited to adjust tomato fruit carotenoidbiosynthesis to the progression of ripening by sensing chlorophyll contents

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Plants detect the presence of nearby vegetation by sensing the spectral composition of light filtered or reflected by neighboring green leaves (i.e. shade). Here we unveil a regulatory mechanism that exploits core components of this inter-plant communication system to modulate intra-plant signaling processes controlling carotenoid biosynthesis during tomato (*Solanum lycopersicum*) fruit ripening. As tomato fruits ripe, they turn from green to red due to chlorophyll loss and carotenoid accumulation. Filtering of sunlight through the flesh of green
fruit causes a self-shading effect that helps to prevent undue production of carotenoid pigments. Chlorophyll breakdown changes the quality of the filtered light and this promotes degradation of phytochrome-interacting transcription factors that directly repress the master gene of the fruit carotenoid pathway, boosting carotenoid biosynthesis as self-shading attenuates. Hence, light-signaling components might have been coopted to finely adjust fruit color during the progression of ripening. Similar mechanisms are expected in other plants
bearing fleshy fruits that lose their green color (i.e. degrade chlorophylls) when ripe as a signal for attracting animals that disperse viable seeds.

OC - 66

Role of Phototropin1 in Fruit Ripening

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The ripening of tomato fruits is a complex and coordinated programme involving diverse processes such as cell wall loosening, enrichment of nutrients, accumulation of sugars and organic acids, enhancement of flavor, colour, etc. Apart from regulation by a genetical hierarchy, a subset of fruit ripening is also regulated by light signaling as negative regulators of phytochrome signaling and photoreceptors like phytochromes and cryptochromes influence carotenoid levels. We now report that the blue light photoreceptor, phototropin also regulates pigmentation and shelf life of tomato fruits besides mediating chloroplast movements, phototropism, leaf movements and stomatal opening. Analysis of fruit development in Nps1 (non-phototropic seedling) mutant impaired in the PHOT1 gene revealed a delayed attainment of mature green stage than wild type after which it ripens normally. The mutant fruits also emitted less ethylene than WT fruits. The Nps1 fruits exhibited 4.7 fold higher lycopene than the wild type fruits with no significant change in β-carotene content. However, no correlation was observed between the carotenogenic gene expression and the carotenoid content, suggesting a post-transcriptional regulation of the carotenoid pathway in the mutant fruits. In addition, the shelf life of Nps1 fruits was significantly enhanced in both on-vine and off-vine conditions than the wild type fruits. Analysis of proteome profiles in Nps1 and WT fruits during ripening revealed 176 differentially expressed proteins, of which proteins mediating carbohydrate metabolism, stress, secondary metabolism and hormone metabolism were the major functional classes. A subset of cell wall related proteins were found to be overexpressed in the mutant fruits which may contribute towards long shelf life and the data would be presented.

OC - 67

Enhancing Photosynthesis in tomato fruit to increase tomato fruit quality

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Fruits are generally regarded as photosynthate sinks as they rely on sugars transported from leaves to carry out the highly demanding processes of development and ripening; eventually these imported photosynthates also contribute to the fruit organoleptic properties. The effect of engineering transcriptional factors GLKs and APRR2 alone or in combination to enhance chloroplast development in fruit has been studied in detail here. As a refinement of previous work with these TFs we have used a developmentally regulated fruit promoter to drive expression of both genes, this resulting in fruits containing more numerous, larger and more highly structured chloroplasts. The chloroplast fruit phenotype showed specific differences in each case which included a synergistic effect when both TF were introduced in combination. The molecular mechanisms underlying the enhanced chloroplast phenotype in those fruits as well as the consequences in ripe fruit quality traits will be presented. Our results suggest additional ways to improve fruit quality by fortifying fruit chloroplasts and plastids. These results together with the variability detected leaf photosynthesis and in fruit Chl content across the tomato germplasm pool has let us to re-evaluate the importance of the contribution of chloroplasts/photosynthesis to fruit development and ripening.

OC - 68

Response of plants to abiotic stress: a story of lipids.

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Light is required for photosynthesis and hence essential for plant growth and productivity. However when in excess, light causes severe stress to the plants amongst other effects by damaging the photosynthetic reactions centers. Due to climate changes, plants have to cope with increasing temperatures often in combination with high light intensity. The photosynthetic light reactions occur at the thylakoid membranes in the chloroplast. The machinery responsible for the photosynthetic activity is composed of protein and lipid components. The lipid components not only include membrane lipid but also pigments (chlorophylls, carotenoids) as well as prenylquinones (plastoquinone, phylloquinone, tocopherol) that are embedded in the thylakoid membrane. Apart from their respective roles in light harvesting and electron transport, carotenoids and prenylquinones have important antioxidant properties and protect plant cells against ROS (reactive oxygen species). In response to environmental change not only chloroplast ultrastructure, photosynthetic complex formation but also the the lipids composition changes. Together these changes reflect remodeling of the photosynthetic machinery taking place in the chloroplast. The aim of my work is to understand the role of carotenoids and prenylquinones in stress acclimation. This poster shows how the combination of light and temperature stress impacts the lipid components of the photosynthetic machinery in tomato, an important member of the Solanaceae family. The results provide new insight on the ability of the photosynthetic machinery to acclimate to the ever-changing environment.

SP - 16

Nicotiana genomics: from plants to genomes

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Nicotiana tabacum (common tobacco) is a major crop species and a model organism, and as a Solanaceae shares significant similarities with tomato, potato, eggplant and pepper. The three most commonly used tobacco types are Flue-Cured (or Virginia), Burley and Oriental, which are traditionally grown and harvested under different agricultural practices in over 120 countries. Tobacco plant stands out as a complex allotetraploid with a large 4.5 Gb genome, a significant proportion of which represented by repeats. As a species, *N. tabacum* (2n=4x=48) evolved through the interspecific hybridization of the ancestors of two South American Nicotiana species about 200,000 years ago, *Nicotiana sylvestris* (2n=24, maternal donor) and *Nicotiana tomentosiformis* (2n=24, paternal donor). Efforts to sequence a reference tobacco genome started almost 15 years ago with the Tobacco Genome Initiative, and several milestones have been reached and made available. A reduced representation genome of the Hicks Broadleaf tobacco variety, a breeding background of many flue-cured tobacco cultivars in use today, was released in 2007 by the Tobacco Genome Initiative. This enabled the publication in 2011 of a genetic map with 2317 markers and 2363 loci generated using an F2 mapping population derived from the intervarietal cross of Hicks Broadleaf × Red Russian. In 2013, a physical map of four bacterial artificial chromosome (BAC) libraries totaling 425,088 clones from Hicks Broadleaf was constructed using KeyGene's Whole Genome Profiling (WGP™) technology. The map obtained consists of 9,750 contigs containing 330,632 BACs, and the calculated genome coverage equals the estimated tobacco genome size. Draft genomes for the diploid Nicotiana species *N. sylvestris* and *N. tomentosiformis* were completed, covering 72-83% of the 2.3-2.6 Gb genomes, and in 2014, draft genomes for three varieties of the tetraploid Nicotiana species *N. tabacum* were published, covering 81-84% of the 4.4-4.6 Gb tobacco genome. These genomes show both the low divergence of tobacco from its ancestor genomes and display microsynteny with other Solanaceae species. We anticipate that these genomes will strengthen the use of *N. tabacum* as a versatile model organism for biotechnology applications.

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Genomic changes generated in natural and synthetic *Nicotiana* allotetraploids : what do transposable elements tell us ?

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Allopolyploids originate from hybridization between divergent genomes associated with chromosome set doubling. Their genomes may undergo a wide range of structural, epigenetic and functional changes. As transposable elements (TEs) are major components of plant genomes, they may play a key role in the genetic and functional modifications produced by the allopolyploidy process.
We assessed the extent of genomic changes associated with TEs in three recent allopolyploid species of the *Nicotiana* genus : *N. rustica*, *N. arentsii* and *N. tabacum* (tobacco), for which it is possible to create *de novo* synthetic hybrids from the extant accessions of parental species. To unravel the extent of TE-associated structural changes, we performed comparative analysis of SSAP (Sequence-Specific Amplification Polymorphism) profiles obtained for six different endogenous TE populations in both natural and synthetic accessions as well as their diploid progenitors. In natural young *Nicotiana* allopolyploids, each TE family displays a specific evolutionary trajectory. The loss of parental bands is the main event and levels of new bands roughly reflect what is known about the dynamics of each TE. TE divergence between progenitors is also strongly correlated with TE-associated restructuring levels, in agreement with the genome shock model. In *Nicotiana* synthetic hybrids, we observed mainly additive profile as expected but also some TE-related genomic restructuring in F1 hybrids, indicative of TE-associated genome reorganization at early hybridization steps.

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Properties of *Nicotiana glauca* as a biorefining feedstock

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Increasing atmospheric CO_2 levels, temperature and raising sea levels has been pressurising societies to increase sustainability in the environment. *Nicotiana glauca* is a potential feedstock for biorefining, it has the advantage of relatively high quantities of long chain hydrocarbons (C_{29}-C_{33}) and a simple plant composition of fatty acids which makes this plant material a potential source for biofuel particularly aviation blended fuel. This species will grow on margin land and will not compete with food production, has a wide range of valuable secondary metabolites and is amenable to genetic manipulation. In this study analyses of the non–polar fraction from *N. glauca* over ten stages of leaf development. The study also looks into summer and winter cultivated crops and metabolite changes at key stages of leaf development. The analysis demonstrate a total 20% of hydrocarbon content in non–polar metabolite composition, of which 94% was hentriacontane. Further analyses showed high levels of phytol, C16:0 palmitic acid, C18:2 cis 9,12 linoleic acid and relatively high carotenoid and sterol content. Meanwhile, comparing summer and winter harvests, a decrease in fatty acids, phytol and phytoene was observed in winter crop. On the contrary, an increase of sterols and most carotenoids was recorded in winter harvest. Overall, no significant changes were observed between summer and winter crops in hydrobcarbon quantities. It is concluded that *glauca* does not require a specific stage or season of harvest for biorefining processes and in addition to biofuels other metabolites and commodities are present that can act as valuable side streams.

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Development and evaluation of a potential bio-refining cascade for *Nicotiana glauca*

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The engineering of biochemical pathways in plants has provided valuable insights into metabolic regulation. This knowledge can now be used to generate renewable sources and products for societal and environmental benefit. In this project, an EU consortium funded through the FP7 programme has developed the tobacco tree (*N.glauca*) as a potential biorefining feedstock. Large scale extraction procedures have been developed for *N. glauca* and further analysis of the fractions and products have been profiled using metabolomics to identify high value products and co-products. Furthermore, the engineering of the isoprenoid pathway in *N.glauca* is underway to enhance the potential of the material as high value compounds and in the search of novel industrial end products.

Crossover, mutagenesis and transformation – from randomness to precise breeding

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Plant breeders rely on tools that they do not control in order to generate genetic diversity. This includes meiotic recombination, mutagenesis and transgenesis. In order to better understand the factors regulating the variable crossover landscape in Arabidopsis, we performed a detailed genetic and epigenetic analysis of 737 crossover events in Arabidopsis. Crossovers were more frequent than expected in promoters. Three DNA motifs enriched in crossover regions and less abundant in crossover-poor pericentric regions were identified. Analysis of epigenetic modifications around the motifs showed, in most cases, a specific epigenetic architecture For example, there is a peak of nucleosome occupancy and of H3K4me3 around the CCN and CTT repeat motifs while nucleosome occupancy was lowest around the A-rich motif. Cytosine methylation levels showed a gradual decrease within ~2Kb of the three motifs, being lowest at sites where crossover occurred. Overall, the crossover motifs are associated with epigenetic landscapes corresponding to open chromatin. Similarly, the distribution of genomic sites of Agrobacterium's T-DNA insertion depended on whether these events were selected for transgene expression and on the epigenetic architecture in the genome. Finally, we show that the CRISPR-Cas system can be used for effective targeted mutagenesis in tomato and preliminary results suggest that it can also be used for targeted crossover induction in tomato. Future work should integrate genetic and epigenetic data in order to achieve efficient genome editing for precise plant breeding.

SP - 17

Towards improved fruit set efficiency using tomato genetic resources.

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National Bioresources Project (NBRP) is a Japanese funding project with an aim to establish centers for collecting, preserving and providing bioresources for scientists worldwide. The University of Tsukuba, as a core institute for NBRP-tomato, has been accumulating bioresources of tomato, a model system of plants bearing fleshy fruits. As of 2015, 16,902 lines of M_3 mutagenized populations and TILLING platforms comprising of 8,448 EMS-derived lines have been developed using Micro-Tom, a dwarf and rapid growth variety. Those resources and platform coupled with next-generation sequencing (NGS), now allow for efficient identification of mutants potentially benefit for breeding as well as genes governing tomato important traits. Fruit set, a developmental process of ovary into fruits, is an important breeding trait since it determines yield and is known to be induced by gibberellin (GA). GA stimulates fruit set via degradation of SlDELLA, a negative regulatory protein and thus loss-of-function in SlDELLA enhances fruit set efficiency. We isolated several mutants showing improved fruit set including multiple alleles of sldella. One of sldella allele sldella-2 showed weaker GA responses than procera, a naturally obtained allele, while these showed higher yield even under high temperatures, suggesting that sldella mutations might be useful for breeding of high temperature tolerance. We also present experimental results of the other mutant analyses where candidate genes have been isolated by SNP infinium array couple with NGS. This work was supported by NBRP and Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry, Japan (grant no. 26013A).

SP - 18

GoldenBraid, a multigene assembly platform for Solanaceae genome engineering and editing

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Synthetic Biology-inspired Modular DNA assembly methods are increasingly implemented in Solanaceae research. Modular cloning facilitates genetic engineering by providing simple rules for the physical composition of multigenic constructs. Thus, standardized building blocks such as promoters, CDS and terminators can be assembled into higher order modules following a discrete set of physical composition rules. Standard genetic modules can be reused for different purposes, exchanged between laboratories and/or employed in combinatorial approaches. Furthermore, modular cloning has encouraged the creation of databases and/or repositories of synthetic genetic elements and the implementation of computer-assisted design. We recently launched Goldenbraid2.0 (GB2.0) (https://gbcloning.upv.es/), a comprehensive web-based tool devoted to modular cloning in Plants. GB2.0 implements an iterative cloning strategy and comprises a database, a repository of exchangeable genetic elements and a set of software tools for assisting in constructs design.
To provide plant community researchers with new reverse genetic engineering tools, we are developing a new version of GB which expands its current capabilities. Among other features, we have GB-adapted all the genetic elements required for CRISPRs-based genome editing and transcriptional regulation, and new software tools for CRISPRs assembly have been incorporated to the public GB resources site. The functionality and the efficiency of CRISPR GB tools was demonstrated in Nicotiana benthamiana using transient expression assays both for genome editing and for transcriptional regulation. The availability of CRISPR GB toolbox will facilitate the application of CRISPR technology to genome engineering in Solanaceae.

SP - 19

Genetic dissection of systemin/jasmonate-mediated systemic defense signaling in tomato

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In tomato (*Solanum lycopersicum*), the peptide signal systemin and the phytohormone jasmonic acid (JA) work together in the same signaling pathway to regulate wound-induced expression of defense-related genes. Grafting experiments by using tomato mutants defective in JA biosynthesis or signaling revealed that the long distance mobile signal in regulating systemic wound response is JA. These results challenge the previous paradigm that systemin is the long-distance mobile signal for wound-induced defense gene expression. Decades of studies in Arabidopsis have illustrated a core JA signaling module consisting of the F-box protein CORONATIN INSENSITIVE 1 (COI1) that forms a functional Skp-Cullin-F-box (SCF) E3 ubiquitin ligase complex, a group of JASMONATE-ZIM domain (JAZ) proteins that function as transcriptional repressors, and the master transcription factor MYC2 that differentially regulates diverse aspects of JA responses.
Here we report the function of the tomato JAZ7 protein in regulating JA- and salicylic acid (SA)-mediated defense responses. We show that, in response to mechanical wound or MeJA treatment, the expression of proteinase inhibitor (*PI*) genes and other JA-responsive genes are largely reduced in *JAZ7-OE* plants, indicating that the JA-dependent immune responses were impaired in these plants. Consistently, *JAZ7-OE* plants show compromised resistance to both insect attack and *Botrytis cinerea* infection. We further show that, SA-induced expression of pathogenesis-related (*PR*) genes was enhanced in *JAZ7-OE* plants. Consistently, *JAZ7-OE* plants exhibit increased resistance to *P. syringae* pv. Tomato DC3000 infection. These results support that JAZ7 acts as an interaction node between JA- and SA-dependent plant immune responses and highlight that the antagonistic effect of JA on SA signaling is controlled at the level of transcriptional regulation.

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Assessing the genetic variation of *Ty-1* and *Ty-3* alleles conferring resistance to Tomato yellow leaf curl virus in a broad tomato germplasm

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Our previous studies focussed on mapping and cloning the TYLCV resistance genes *Ty-1* and *Ty-3*; both genes are derived from *Solanum chilense* and were shown to be allelic. They code for a RNA-dependent RNA polymerase (RDR) of the RDRγ type defined by a DFDGD catalytic domain. In this study we first fine-mapped the TYLCV resistance in *S. chilense* LA1932, LA1960 and LA1971. Results showed that chromosomal intervals of the causal genes in these accessions overlap and cover the region where *Ty-1/Ty-3* is located. Further, VIGS was used to silence *Ty-1/Ty-3* in tomato lines carrying TYLCV resistance introgressed from *S. chilense* LA1932, LA1938 and LA1971. Silencing *Ty-1/Ty-3* compromised the resistance in lines derived from *S. chilense* LA1932 and LA1938. The LA1971-derived material remained resistant upon silencing *Ty-1/Ty-3*. Further, we studied the allelic variation of the *Ty-1/Ty-3* gene by examining cDNA sequences from nine *S. chilense*-derived lines/accessions and more than 80 tomato cultivars, landraces and accessions of related wild species. The DFDGD catalytic domain of the *Ty-1/Ty-3* gene is conserved among all tomato lines and species analysed. Compared with the susceptible *ty-1* allele, the *Ty-1/Ty-3* allele is characterized by three specific amino acids shared by seven TYLCV-resistant *S. chilense* accessions or derived lines. Thus, *Ty-1/Ty-3*-specific markers can be developed based on these polymorphisms. Elevated transcript levels were observed for all tested *S. chilense RDR* alleles (both *Ty-1* and *ty-1* alleles), demonstrating that elevated expression level is not a good selection criterion for a functional *Ty-1/Ty-3* allele.

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Tomato responses to salt stress and powdery mildew combination: genetic resistance and the effect of salt stress intensity

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As climate change progresses, the spread and intensity of abiotic and biotic stressors is expected to increase, which increases the chances of stress combinations reducing crop growth and yield. We examined the effects of combined salinity and disease by evaluating the resistance to powdery mildew, to salt stress (100mM NaCl), and to both stresses combined in tomato, using the S. habrochaites LYC4 introgression line (IL) population. The IL population segregated for both salt stress tolerance and powdery mildew resistance. Using SNP array marker data, QTLs were identified for salt tolerance as well as Na+ and Cl- accumulation. 100mM NaCl increased the susceptibility of the population to powdery mildew in an additive manner. Phenotypic variation for disease resistance was reduced under combined stress as indicated by the coefficient of variation (CV). No genetic correlation was found between disease resistance and Na+ and Cl- accumulation under combined stress. Most genetic loci were specific for either salt stress tolerance or powdery mildew resistance. We evaluated the responses to combined stress of selected ILs under variable salt stress levels (50, 100 and 150mM NaCl), and observed increased powdery mildew susceptibility under mild salt stress (50mM), which was accompanied by accelerated cell death-like senescence. On the contrary, severe salt stress (150mM) reduced the disease symptoms. Na+ and Cl- accumulation in the leaves across the different salt stress levels was linearly related to the decreased pathogen growth under severe stress. Exceptional resistance-type specific responses were observed for the powdery mildew resistant NILs Ol-1, ol-2 and Ol-4. Gene expression analysis indicated increased expression of ethylene/jasmonic acid pathway genes in particular under combined salt and powdery mildew stress. The increased understanding of combined stress tolerance genetics and the underlying molecular mechanisms will aid to efficiently breed for tomatoes that exhibit a high level of resistance in a changing climate.

SP - 20

Plant susceptibility (S) genes in resistance breeding: promises and limitations

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Resistance and susceptibility are opposite sides of the same coin. Most studies have for a long time focused on the resistance side, in search for plant resistance (R) genes. Based on studies of effector-triggered susceptibility and by looking from a different angle into non-host resistance, we proposed that disabling plant susceptibility (S) genes may help to achieve durable and broad-spectrum resistance in crops (Pavan et al., Mol Breeding (2010) 25:1–12). As a proof of concept, we have silenced (via RNAi) tomato and potato orthologs of several S-genes identified in Arabidopsis, resulting in resistance to powdery mildew, late blight and grey mold. Our results suggest that the S-genes identified in Arabidopsis are conserved for their function as susceptibility factors to a broad range of pathogens in other plant species. Further natural and induced mutations have been identified for certain S-genes by (in silico) allele-mining in plant germplasms and a tomato EMS population. Therefore, it is very promising to deploy loss-of-function mutations of S-genes for durable resistance in controlling plant diseases. Although fitness cost may be a disadvantage of using altered host S-genes, it is specific to plant species and can be moreover overcome by both classic (e.g. searching for allelic variants) and advanced approaches (e.g. editing S-gene).

OC - 74

Understanding the mechanisms of bacterial wilt disease for future sustainable resistance breeding in Solanaceae

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Our research group is focused on the study of the soil bacterial plant pathogen Ralstonia solanacearum. This devastating bacterium provokes the bacterial wilt disease on a wide range of host including all Solanaceae crops. Its persistence in the soil makes it particularly hard to eradicate.
Our first aim is to better understand the virulence determinants of this bacterium. I will present our current understanding of the virulence cornucopia present in the worldwide diversity of R. solanacearum.
Our second goal is to identify the tomato proteins and pathways targeted by the virulence determinants that the bacterium directly injects into the cells of its hosts. These candidate targets are then studied by a set of functional genomics approaches in order to evaluate their contribution to the disease.
Finally, the combination of the knowledge of the diversity of the virulence determinants together with the identification of plant targets essential for the establishment of the disease should provide means for sustainable bacterial-wilt resistance breeding in a wide range of Solanaceae crops.

OC - 75

Monocot and dicot MLO powdery mildew susceptibility factors are functionally conserved in spite of the evolution of class-specific molecular features

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Specific members of the plant Mildew Locus O (MLO) protein family act as susceptibility factors towards powdery mildew (PM), a fungal disease threatening many cultivated species. In order to obtain PM resistant phenotypes, a novel breeding strategy was proposed based on the selective inactivation of *MLO* susceptibility genes across cultivated species. Phylogenetic analysis and sequence alignment are used to predict possible orthologs of MLO proteins. Interestingly, both monocot and dicot MLO proteins involved in PM susceptibility are phylogenetically divergent.
Our results showed that monocot and dicot MLO susceptibility proteins evolved class-specific amino acids. To test whether these different molecular features are specifically required by PM fungal species attacking either one or the other class of Angiosperm, we transformed a resistant tomato mutant impaired for the endogenous *SlMLO1* gene with heterologous *MLO* susceptibility genes from the monocot barley and the dicots pea and tobacco.
In all the three cases, we observed restoration of PM symptoms after the inoculation with the adapted PM *Oidum neolycopersici* and increased penetration with respect to the non-adapted PM *Blumeria graminis* in epidermal cells of transgenic tomato plants. Finally, we provide an overview of MLO protein molecular features predicted to play a major role in PM susceptibility, including a novel mutation occurring in tobacco *NtMLO1*.
In conclusion, our study offers insights on the evolution and function of *MLO* genes involved in the interaction with PM fungi and points out specific regions of the MLO protein that represent ideal targets for approaches of reverse genetics in breeding programs.

OC - 76

Identification of the Receptor for Bacterial Cold Shock Protein from Tomato

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Perception of Microbe Associated Molecular Patterns (MAMPs) by Pattern Recognition Receptors (PRRs) is an important step of innate immune system in plants. Some MAMP/ PRR pairs such as flagellin/FLS2 and EF-Tu/EFR are well studied but several MAMPs identified long time ago remain orphan with respect to their receptors. The peptide csp22 of bacterial Cold Shock Protein (CSP) was identified as a MAMP acting in some of the *Solanaceous* plants more than a decade ago, yet its receptor remained unknown. Using a forward genetic approach, the locus determining the sensitivity of tomato to csp22 was mapped, which encodes a leucine-rich repeat receptor like kinase. This protein was named **Co**ld Shock Protein **Re**ceptor (CoRe), since it was found to act as a specific, high-affinity binding site for csp22. Heterologous expression of CoRe in *Arabidopsis thaliana*, a plant unable to sense csp22, conferred responsiveness to csp22 and provided enhanced resistance against the hemibiotrophic bacterium *Pseudomonas syringae*.

OC - 77

Comparative Proteomic Analysis of Tomato Roots Colonized by *Verticillium dahliae*

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Verticillium species are destructive vascular wilt fungi with worldwide distribution, causing severe losses in crop yield and quality. These soil-borne pathogens colonise the plant root surface in response to root exudates, penetrate the cortex and endodermis, and spread systemically through conidia transported by the transpiration stream in the xylem. While a large body of physiological and biochemical alterations in the host are reported, the cellular effects of pathogen colonisation on the host's root are still not fully clarified. Here we report on the time-resolved analysis of the tomato root proteome in response to fungal colonisation. Tomato (*Solanum lycopersicum* cv. Hildares) was grown in quartz sand and inoculated with *Verticillium dahliae* at the two-leaf stage. Roots were harvested at seven, 14 and 21 days after inoculation. In order to identify proteins related to the fungal spread at the different time points, a subsequent proteome analysis by two-dimensional differential in gel electrophoresis (2-D DIGE) was initiated on samples from three independent experiments. Hierarchical clustering and principal component analysis were applied to interpret the data set. First results of protein identifications using MALDI-TOF MS/MS from the comparison of treated and non-treated plants as well as from the time-course analysis of fungal spread are presented.

SP - 21

Hormonal and metabolic regulation of abiotic stress responses in tomato

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Abiotic stresses like drought and salinity have a negative impact on tomato (*Solanum lycopersicum* L.) productivity by inducing premature senescence in the photosynthetic source tissues of the plant and by reducing the number and growth of the harvestable sink organs by affecting the transport and use of assimilates between and within them. It has been hypothesized that yield stability implies the maintenance or increase of sink activity in the reproductive structures, thus contributing to the transport of assimilates from the source leaves through changes in sucrolytic enzymes and their regulation by phytohormones. Classical and functional genetic and physiological approaches have been integrated to study the influence of metabolic and hormonal factors on tomato fruit sink activity, growth, and yield under salt and water stresses. The overexpression of a cell wall invertase in fruits and leaves and *de novo* cytokinin biosynthesis in the roots similarly increases stress tolerance and water use efficiency by inducing sink metabolism in leaves and fruits, limiting water consumption and maintaining photosynthetic and source activity. This is possibly related to an increase of sink metabolism due to both (i) increase in sucrolytic activities (sucrose synthase, cell wall, vacuolar and cytoplasmic invertases) and *trans*-zeatin concentration, and (ii) a decrease in the sink inhibitor and senescence-inducing ethylene precursor 1-aminocyclopropane-1-carboxylic acid. New functional evidences about the role of metabolic and hormonal inter-regulation of local sink processes in controlling sink and source activities, growth, and yield in tomato plants provide new opportunities to maintain sustainable crop production under changing environmental conditions.

OC - 78

Carbon partitioning in potatoes under drought stress

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Potato (*Solanum tuberosum*) is an important crop species consumed all over the world, but it is generally sensitive to drought conditions. In view of the huge yield losses resulting from drought stress, the drive for improved drought tolerance in potato has gained global research and agricultural interest. One of the major physiological processes affected by drought stress is carbon partitioning: the plant's choice of where to allocate its photoassimilates under stress is strongly affecting yield in crops. Carbon partitioning and its relation to yield involve many processes including photosynthesis, sucrose metabolism, transport of metabolites, and starch biosynthesis. These were studied in the greenhouse from 2012 – 2015 using potato cultivars with contrasting drought responses. Our results indicate that one of the most severe effects of drought stress is the arrest of stolon differentiation and formation of tubers. Our phenotypic studies also point to some physiological traits that affect photosynthesis and eventual tuber yield. Multidisciplinary studies of photoassimilate metabolism and transport using MRI to visualize xylem and phloem flow, enzymatic assays and gene expression analyses to measure activities of sucrose-metabolizing enzymes in various source and sink tissues in combination with phenotypic assessments, highlight the various tissues prioritized by the plant for assimilate transport during drought stress, and give indications of what distinguishes drought tolerance and sensitivity of cultivated potato. Some of the key enzymes and genes studied may be inclusive breeding targets for drought tolerance in potato.

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New potato genetic elements involved in response to drought stress

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Two pairs of Polish closely related potato varieties (common one parent), differing strongly in terms of tolerance to water deficiency were selected. In each pair there is a cultivar sensitive to drought whereas the other one is drought tolerant. Despite close relation within each pair, there is no close relation between the pairs. Detailed morphological and physiological analysis showed differences between the varieties with benefit for drought tolerant varieties in: stomatal and trichomes density, ABA-dependent stomatal closing, cuticle thickness and relative water content.

Drought experiment and transcriptome sequencing at different time points upon drought and in control conditions were carried out. Differentially expressed genes in drought-tolerant varieties in comparison to drought-sensitive varieties were identified (47 and 174 upregulated, 64 and 142 downregulated genes in each pair, respectively). We found well-known drought responsive genes as well as unknown ones. Identified genes differ between the pairs of varieties indicating two different mechanisms of drought tolerance. The results were validated by RT-PCR analysis. Additionally, genes with stable expression upon drought in all four cultivars studied were selected and confirmed by RT-qPCR.

New drought responsive potato microRNAs and their target genes were identified and analysed. Some of these microRNAs differ in their expression levels between the cultivars that are drought sensitive and tolerant.

The described non-standard approach with the use of closely related potato cultivars that extremely differ in their tolerance to drought allowed us to identify new genetic elements involved in plant response to drought stress.

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Genotype by watering regime interaction in cultivated tomato: from phenotypes to genes.

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In the next decade water will be limiting crop production, in particular in Mediterranean regions. Studying genotype x water regime interactions is needed to improve plant adaptation to drought. In response to environmental constraints, plants can change their phenotypes (at molecular, physiological and morphological levels). In Tomato, extensively grown in Mediterranean regions, first studies have shown genetic variability in the response to drought, but few genes/QTLs have been identified and mostly in wild related species. Studying water deficit in this crop is of particular interest since a mastered water deficit can stimulate metabolite production, increasing plant defenses and concentration of compounds involved in fruit quality at the same time. We analyzed two populations: recombinant inbreed lines (RILs) and unrelated cherry tomato accessions, grown in greenhouse under two watering regimes. We assessed a large genetic variability and highly significant genotype x water regime interactions, for several plant and fruit traits, in the two populations. Large fruit accessions showed high sensitivity to drought. The two panels were genotyped with large sets of SNP and quantitative trait loci (QTLs) were identified, combining linkage and association mapping. 20% of the QTLs were interactive between the watering regimes, mostly with antagonist effects according to treatment. Analysis whole genome of gene expression in young leaves from the RIL parents provided interesting candidate genes under interactive QTLs.

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Regulation of pollen thermotolerance in tomato (*Solanum lycopersicum*)

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Male gametophyte development is considered as the most sensitive stage of the plant life cycle to heat stress. Mild chronic stress or short exposure to high temperatures can lead to loss of pollen competitiveness and abortion. This sensitivity has been mainly attributed to the limited capacity of meiocytes and developing microspores to induce an adequate heat stress response [1]. In plants, heat stress response is mediated by the activity of heat stress transcription factors (Hsfs). Hsfs enhance the transcription of heat shock proteins (Hsps) as well as other genes involved in protection of protein homeostasis and cellular functions during stress [2]. In tomato, HsfA2 accumulates under stress conditions contributing to the strong upregulation of numerous genes [3]. Analysis of gametophytic and sporophytic cells derived from flowers of HsfA2-RNAi plants exposed to heat stress revealed that HsfA2 is not essential for the induction of Hsps, due to the compensation by other members of the Hsf gene family. However, a short heat stress treatment caused a significantly stronger reduction in pollen release and viability in transgenic plants compared to wild type. Pollen developmental stage-specific analysis revealed that HsfA2 is required for the accumulation of several heat stress induced Hsps during pollen meiosis and microspore formation under physiological conditions. We show that HsfA2 is an essential component of the regulatory network controlling the developmental priming of pollen cells. Identification of cultivars or wild accessions with higher levels of HsfA2 could be used as a source for improving pollen thermotolerance in breeding programs.

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Abstracts

Posters

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P-I-1

Modulation and controlling processes at the mycorrhizal interface in context of plant nutrient and carbon balance

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The arbuscular mycorrhizal symbiosis is formed by ubiquitous soil fungi of the phylum *Glomeromycota* and is found in the vast majority of vascular plants. Recent research shows that the control in this symbiosis is bidirectional. Plants are able to distinguish between different species of fungi and they can discriminate between them, rewarding the more cooperative one with additional carbohydrates. Likewise, the fungus can hold back nutrients depending on the amount of carbon supplied by the plant. These observations were made for phosphate and nitrogen so far. Yet the controlling processes remain unknown. Therefore we studied the expression patterns of the phosphate transporter genes LePT4 and LePT3, as well as the sucrose transporter gene LeSUT1, from tomato in interaction with the fungal symbiont *R. irregularis* in a split root experiment. All three transporter genes are known to be activated during mycorrhizal symbiosis or have a higher expression rate in mycorrhizal compared to non.-mycorrhizal plants. First results already indicate a local regulation of LePT4 and LePT3 and reject a systemical regulation. In a second experiment, the fungus had access to different sources of the same nutrient. The results imply that the source of a nutrient might also influence the expression pattern of these transporter genes. That is why different phosphate concentrations as well as different sources of the nutrient will be tested in further split root experiments to determine which factors are mainly involved in the regulation process and to help identify who is in control in this symbiosis.

P-I-2

UVC Irradiation in Mutational Breeding of Potato (*Solanum tuberosum)*

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UV irradiation was chosen for induction variability of *tbr* cv. Herby with aim to obtain interesting colour of tuber flesh. The leaf protoplasts were used for mutagenesis. They were irradiated for 2, 4 and 6 minutes (wave length 240 nm, dosage 370 μW cm^{-2}) and cultivated into organogenic calli that gave 16, 22 and 34 individuals, respectively. The first treatment generated only low differences compared to control. The 4 and 6 minutes´ variants induced higher variability and four genotypes with exceptional flesh colour were selected for more detailed greenhouse and field assessment. Herby 4´/7, Herby 4´/15 and Herby 6´/9 kept leaf deformations so they were excluded from experiments next season. All tested genotypes induced flowering. The field assessment of attack of *Phytophthora infestans* showed the similar level of resistance to this pathogen as *tbr* cv. Herby. Notable nibbling the leaves by *Leptinotarsa decemlineata* was not noticed. The mutants and cv. Herby alike had very low level of resistance to PVY and PVS. All genotypes had different flesh colour, the other tuber characteristics were quite similar to *tbr* cv. Herby except Herby 4´/15 with primary yellow colour of skin. Mutant genotypes did not reach the yield of *tbr* cv. Herby. Reciprocal sexual breeding (*tbr* cv. Herby × Herby 6´/29 and vice versa) has not given the offspring yet.
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P-I-3

Genome Wide Analysis of Aquaporin, Sugar Transporter and ABC Transporter in Tomato

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Genome of tomato had been sequenced and precise gene prediction of tomato is provided. However definition of gene families and classification of their members of tomato are far poor than those of Arabidopsis. Information of gene families is fundamental information to clarify gene functions and their physiological roles in tomato growth. Our interests are functions of transporters in tomato. In this study, we focused on aquaporins, sugar transporters and ABC Transporters. Aquaporins are important not only for water transport but also as channels, which diffuse ammonia, urea, boron, silicon, CO_2, H_2O_2 and so on. Sugar transporters play roles in sugar transports from at organelle level to at organ level. ABC transporters transport wide range substrates, including flavonoids, terpenoids, alkaloids, malate, wax, auxin, abscisic acid and strigolactone. We determined gene members of aquaporin family, sugar transporter family and ABC transporter family in tomato. As a result, 47 genes encoding aquaporins, 52 genes encoding sugar transporters, 170 genes encoding ABC proteins (most are ABC transporters and the others are soluble proteins) are found. We annotated and added valuable information of them, including exon-intron structures and gene expressions in various organs or tissues (Reuscher et al. 2013, 2014).

P-I-4

A cascade of arabinosyltransferases controls shoot meristem size in tomato

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Shoot meristems of plants are made of stem cells that are continuously replenished through a classical feedback circuit involving the homeobox gene WUSCHEL and the CLAVATA (CLV) signaling pathway. In CLV signaling, the CLV1 receptor complex is bound by CLV3, a secreted peptide modified with sugars. However, the pathway responsible for modifying CLV3, and its significance for CLV signaling, is unknown. Here we show that tomato inflorescence branching mutants with extra flower and fruit organs due to enlarged meristems are defective in arabinosyltransferase genes. The most extreme mutant is disrupted in a hydroxyproline O-arabinosyltransferase that can be rescued with arabinosylated CLV3. Weaker mutants are defective in arabinosyltransferases that extend arabinose chains, indicating CLV3 must be fully arabinosylated to maintain meristem size. Finally, we show that a mutation in CLV3 increased fruit size during domestication. Our findings uncover a new layer of complexity in the control of plant stem cell proliferation.

P-I-5

Influence of nitrogen supply on plant growth promoting capacity and transcriptional responses of *Kosakonia radicincitans* in tomato

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Plants benefit from the intimate association with plant growth promoting rhizobacteria (PGPRs). A tailored application of PGPRs is required for the successful use of PGPRs as growth promoters in cash crops under various environmental conditions. *Kosakonia radicincitans* can exert beneficial effects on plant development and growth in tomato. The bacterium shows chemotactic affinity to plant roots, and successfully competes with the native microflora. Inoculation experiments revealed the ability of the bacterium to colonize plant roots. Fixation of biological N_2, *in vitro* production of phytohormones such as auxines and cytokinins, and solubilization of P-compounds have all been demonstrated for
K. radicincitans. To test whether and how the bacteria affect plant performance under diverse nitrogen nutrient regimes, we chose tomato as a model system, and inoculated seeds with *K. radicincitans*. We elucidated the influence of plant N-fertilization on growth-promoting capacity, the bacterial root colonization and the transcriptional responses by PGPR *K. radicincitans* in tomato. We found a correlation between nitrogen fertilization and the intensity of growth enhancement by *K. radicinictans* in tomato plants. While detailed regulatory processes remain unclear we suppose that adequate plant nutrition nitrogen status is necessary to adjust the most efficient plant growth promotion by *K. radicincitans*.

P-I-6

Functional role of *MACROCALYX* as key regulator of tomato inflorescence development: new evidence from the characterization of *vegetative inflorescence* (*vin*) mutant

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The *vegetative inflorescence* (*vin*), a new recessive mutant affecting reproductive development, was identified from the screening of a T-DNA mutant collection generated from the tomato (*Solanum lycopersicum* L.) cultivar Moneymaker. The *vin* mutant developed indeterminate inflorescences that revert to a vegetative growth after the production of two or three flowers. Moreover, *vin* plants developed flowers with leafy sepals and incomplete abscission zone, and its flowering time was delayed compared to wild type. The molecular results showed that a single copy of T-DNA was inserted into the promoter region of the *MACROCALYX* (*MC*) gene; this result together with expression analyses and complementation test confirmed that *vin* is a new knock-out allele of *MC*. Double mutant combinations between *vin* and *jointless (j)* and *single flower truss (sft)* mutants, both affected in inflorescence architecture, were generated for a better understanding of the genetic basis of tomato inflorescence development. The results showed that *MC* has pleiotropic effects on the reproductive phase, and that it interacts with *SFT* and *J* to control floral transition, inflorescence fate, sepal identity and pedicel abscission zone. Taken together, our results provide new evidence about *MC* function as part of the genetic network regulating the development of tomato inflorescence meristem. In addition, *MC* could be involved in regulatory loops together with *SFT* and *J,* and such a MC/J dimer could somehow interact with SFT and other factors to fine-tune the inflorescence development pathway in tomato.

P-I-7

Characterization of the *Curly Leaf* Tomato Mutants and its Role in Leaf Morphogenesis

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As a model plant in the Solanaceae family, research in tomato has received much attention including organ and fruit development researches. Formation of a normal leaf is a complex process that involves the initiation and differentiation of leaf primordia from the shoot apical meristem. The cell division and abaxial-adaxial polarity are the keys of growth of leaf. However, how the polarity is maintained remains unclear. We are characterizing six lines of *curly leaf* (*curl*) tomato mutant which dorsoventrally impaired of leaf flatness, exhibiting severe upward bend on transverse axis. The objectives of the this study are (1) to characterize morphology, hormone, and cytology of the *curl* mutants, (2) to investigate the responsible gene controlling the mutant phenotype and to characterize its function in leaf morphogenesis. Segregation analysis and allelism test had proved that occurred mutation was monogenic recessive and all mutants were allelic. Map-based cloning had demonstrated that mutation is located in short arm of chromosome 9. The *curl* produced impaired leaf curvature, along transverse axis with high extent. By contrast, longitudinal axis remained flat. The upward curvature was initiated from the tip of leaf, followed by the middle and the basal area. In the mature leaves, all leaf had turned to curve, the highest extent was observed in the middle of leaves. In addition, the *curl* mutants showed narrower leaf and shorter petiole. What is gene mutated, cell division, adaxial-abaxial polarity, and auxin response will be investigated to dissect the complicated process of leaf morphogenesis of the *curl* mutants.

P-I-8

Isolation of A New Gene responsible for pistil and fruit development

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Tomatoes show cultivar diversity and are produced worldwide. Tomato fruit shape is builded up from pistil or carpel, although the molecular mechanism for carpel development remains greatly elusive. This research aims to uncover the genetic basis of carpel formation and development using a novel mutant designated *fasciated carpel 2* (*fcc2*), which was isolated from EMS-mutagenized populations in the background of Micro-Tom, a dwarf and rapid growth cultivar. Morphological analysis showed that the *fcc2* mutant bore abnormal fruits with fasciated pericarps with forming additional layers of ovary wall (carpels) at anthesis stage. Scanning Electron Microscope (SEM) analysis showed the two layers of carpel formation was evident and the carpel number was increased in the *fcc2* compared with WT at 1 mm bud stage. Genetic analysis indicated that the responsible gene for the *fcc2* mutant was monogenic recessive. Map based positional cloning and whole genome sequence analysis by the next generation sequencing lead us to identify one candidate gene for the *fcc2* mutant. Next, we produced transgenic tomato in which *FCC2* expression level was downregulated ($FCC2^{RNAi}$). The phenotypes of $FCC2^{RNAi}$ was similar to the original the *fcc2* mutant; twofold carpels formation was evident. Our results demonstrated that isolated gene was responsible gene for the *fcc2* mutant. Our results could provide some new knowledge of carpel formation and development of tomato. This work was supported by JSPS KAKENHI Grant Numbers 23780001 and 221S0002 and Program to Disseminate Tenure Tracking System.

P-I-9

Towards a better understanding of endoreduplication in tomato fruit growth

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Fruit organogenesis results from the coordination between cell division and cell expansion. Cell divisions determine the final number of cells inside the fruit, while cell expansion mainly determines the final fruit size. In tomato fruit, the increase in cell size is associated with the increase in nuclear DNA content according to the endoreduplication process. The endoreduplication cycle is an altered cell cycle, where the mitosis is bypassed, resulting in continuous DNA replication without cytokinesis. The increase in nuclear DNA content thus increases the nuclear volume, which in turn drives cell enlargement according to the "karyoplasmic volume ratio" theory Even if endoreduplication is proposed to have such a role in cell size and fruit size determinations, the precise role of endoreduplication during fruit growth remains to be fully elucidated.
With the aim to investigate the role of endoreduplication during fleshy fruit growth, we selected tomato mutants altered for fruit growth from the Micro-Tom EMS mutant collection developed in our laboratory. The fine physiological and cellular characterization of these mutants revealed various patterns of relationship between fruit size, pericarp thickness, cell size and cell ploidy. We demonstrated a correlation between endoreduplication and fruit growth alterations observed in these mutants. Then, we characterized genetically these Micro-Tom mutants in view to determine whether the locus (or loci) is (or are) responsible for the altered phenotype. This work aims at providing new insights in the understanding of the role of endoreduplication in tomato fruit growth.

P-I-10

The brassinosteroid signalling transcription factor BIM1 is involved in fruit and plant development in tomato

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Early fruit development has important effects on the sensory and nutritional quality of the fruit. Regulatory genes involved in early fruit development can contribute to the control of final fruit size and composition. To identify candidate genes involved in early fruit development, target transcription factors common to tomato and grape were screened by mining public sequences and microarray database. Here we report the characterization of a tomato bHLH transcription factor SlBIM1a, possibly involved in early fruit development. Overexpression of *SlBIM1a* gene in Micro-Tom tomato caused extreme dwarfism and rugose dark-green leaves, alike the brassinosteroid-deficient mutants. Transgenic fruits were smaller than those of wild-type plants and had no round shape, thin pericarp and less bright surface. In contrast, RNAi silenced plants had thick pericarp and more round-shaped fruit compared to wild-type plants. Mean cell surface area of pericarp tissue and its DNA ploidy level were higher in RNAi plants. Interestingly, transcriptome analysis indicated that genes involved in biotic stress responses are up-regulated in *SlBIM1a*-overexpressing plant and the up-regulated genes include tomato homologs of BZR1/2 target genes which are repressed by BZR1/2. In addition, we found that SlBIM1a can interact with the BZR1/2 homolog protein 1 (BZH1) and localized in nuclear bodies. These results suggest that SlBIM1a may negatively regulate fruit and plant development via impairing SlBZH1 in tomato.

P-I-11

Modeling inflorescence development in tomato

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Current research in evolutionary developmental biology aims at revealing mechanisms that account for diversity in inflorescence architecture among flowering plants. We therefore combined unifying concepts to develop a kinetic model of the tomato inflorescence development. The model is based on the maturation kinetics of the successive meristems that elaborate the inflorescence and create a zig-zag patttern. We next exploited the model to explore the diversity of morphotypes that could be generated and matched them with existing mutant phenotypes. This approach allowed us to propose a model of the genetic network controlling development of the primary inflorescence in tomato. Functional comparison with the genetic network disclosed in Arabidopsis highlights differences due to the sympodial patterning of tomato.

P-I-12

Convergence of miRNA-associated pathways and GA in tomato development: roles of miR156- and miR159-regulated pathways in vegetative architecture and fruit development

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The formation and subsequent outgrowth (branching) of axillary buds are key factors that control both biomass production and inflorescence number. In turn, inflorescence and further flower development determines fruit formation and fruit set. All these developmental processes involve phytormones and microRNA-based gene regulation. The natural tomato *procera* mutant (loss of function of *LeGAI*, which encodes a gibberellin or GA DELLA repressor) exhibits altered vegetative branching and inflorescence development as well as seedless fruits. In Arabidopsis, DELLA proteins repress the activity of SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SBP) and GAMYB transcription factors, regulating GA signaling. Interestingly, *SBP* and *GAMYB* genes are also negatively regulated by the microRNAs miR156 and miR159, respectively. Here, we sought to evaluate the roles of miR156/*SBP* and miR159/*GAMYB* nodes in tomato vegetative architecture and fruit development as well as their interactions with GA and *LeGAI*. Tomato transgenic plants overexpressing miR156 and miR159 display higher branching and flower defects. Conversely, MIM159 tomato plants (with low levels of miR159) show low branching. Interesting, alterations in both miR156- and miR159-regulated pathways affect tomato fruit development as well. For instance, miR156-overexpressing plants display high-locule number fruits and fruits containing meristem-like structures. Seedless fruits are generated by miR159-overexpressing tomato plants. Moreover, phenotypic and molecular analyzes of double mutants perturbing both *LeGAI* and miRNA-regulated pathways indicate a convergence of DELLA, GA, and miR156/*SBP* node in regulating tomato vegetative and fruit development. Supported by FAPESP.

SESSION II -Biodiversity		
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P-II-1

Modelling the population history using population genomics : the tomato domestication as a case of study

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A bottleneck is commonly associated to the study of crop domestication, in which a population experiences a drastic reduction in size and nucleotide diversity. The population genomic era offers new opportunities to document this scenario through the study of the site frequency spectrum (SFS), a powerful method for summarizing genomic data at the genome-wide level. Using a diffusion approximation approach (Gutenkunst et al., 2009) to model SFS, we estimated the demographical history of a major crop, the cultivated tomato (*Solanum lycopersicum*) and its wild relative (*S. pimpinellifolium*). We compared the observed 2 dimensional SFS, obtained from 20 individuals and ~84,500 SNPs markers, to the modelled ones, obtained from four different scenarios. Variants were polarized to ancestral and derived alleles based on the eggplant outgroup sequences. Assuming a mutation rate of 3×10^{-9} and a generation time of 1 year, our results suggest that in the best fitting model, population split occurred ~3750 years ago (95%CI: 2985-4552 yrs), wild tomatoes experienced a dramatic bottleneck (effective population size: -87%; reduction of nucleotide diversity: -63%) that occurred ~420 years ago (95%CI: 337-541 yrs). After this bottleneck, migration rate, from cultivated to wild population, was estimated to less than ~1 migrant per generation. Although, 3.1% of the genes derived from neutral expectations suggesting evolutionary forces acting on. These results demonstrate the power of the approach and provide estimates of demographical parameters that fit previous studies of the tomato domestication (Blanca et al., 2015).

P-II-2

Potato germplasm collection in the Czech Republic

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In the Czech Republic bank of potato (*Solanum ssp*.) genetic resources works in the Potato Research Institute Havlíčkův Brod. The institute is in charge of this activity in the framework of the National Programme of Conservation and Utilization of Plant Genetic Resources and Agro-biodiversity and it is the only organization in the Czech Republic with the long-term engagement in this objective. The bank's mission is an assembly, evaluation, documentation, maintenance and delivery of potato genetic resource accessions. The collection involves 2.497 accessions maintained in an *in vitro* culture and divided into six sub-collections (*Solanum tuberosum* varieties, *Solanum tuberosum* tetraploid hybrids, dihaploids and diploids, cultivated *Solanum* species, wild *Solanum* species, interspecific *Solanum* hybrids). Derived information is a part of the genetic resource database maintained in the Czech Republic, which could be found on the website http://www.genbank.cz/genetic/resources and from June 2015 also on https://grinczech.vurv.cz/gringlobal/search.aspx. The data are also accessible on the website http://europotato.org. The long-term maintenance is based on slow-growth culture and *in vitro* tuberization. Microtubers are induced using appropriate media and modified culture conditions. Chitting microtubers after dormancy period or surviving stem segments are subjected to the regeneration passage onto new media in 14-18 months. A revitalization and valorization program is running in the gene bank with the focus on virus infection eradication and screening of maintained material for presence of quarantine viruses, viroids and bacteria, complying with the EU directives.

P-II-3

Characters of leaf epidermis as a contribution to the taxonomy of *Brunfelsia* (Solanaceae)

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A study of leaf anatomy of the epidermis of 25 species of *Brunfelsia,* belonging to the sections *Brunfelsia*, *Franciscea* and *Guianensis*, was carried out in order to contribute to the systematic of the genus, as well as provide subsidies to its characterization. The analysis followed the usual techniques of anatomy for optical and scanning electron microscopy. The measures of the stomata were taken with the aid of ANATI QUANTI 2.0 Program, and Test-T was applied. All species showed hypostomatic epidermis, and sinuous anticlinal walls. Different patterns of anticlinal walls were present in *B. densiflora* and *B. lactea* (section *Brunfelsia*), with straight to curved anticlinal walls, and in two species of the section *Franciscea* (*B. obovata* and *B. rupestris*), with straight walls. Species of the section *Brunfelsia* showed anisocytic and anomocytic stomata, differing from the sections *Guianensis* and *Franciscea* with anisocytic, anomocytic and paracitic stomata, occurring simultaneously. *Brunfelsia mire* and *B. rupestris* (section *Franciscea*), and *B. guianensis* (section *Guiananesis*) showed no anisocitic stomata. The measures of the stomata ranged from 32.09 ± 0.66 and 51.02 ± 4.89 μm without a direct relationship with the sections. SEM observations showed ornate cuticles, with quite diversified striated pattern, which was distinctive and diagnostic for the studied species. The indument, with simple and uniseriate trichomes, was characteristic for species of the section *Franciscea*. The set of characters of leaf epidermis provides subsidies to be used as a tool to the taxonomy of *Brunfelsia* and the delimitation of their species, especially the ornamentation of striated cuticle. (Financial support: CAPES and CNPq).

P-II-4

Profiling morphological, physical and biochemical characteristics of Solanaceae species of Uganda

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Farmer saved seed of Uganda‘s indigenous Solanaceae plants was collected, regenerated, multiplied and characterized. 80 accessions were planted in completely randomized block design and data on morphological and physical characteristics at seedling, vegetative and reproductive stages collected weekly. Plant descriptor protocols were adapted from CABI (*S. lycoperscon*, *C. annum*, *Brassica olereaceae*) and AVRDC (Solanaceae family). The color scale from CABI was singularly used. At 6 weeks after planting, leaves and later fruits were harvested and taken for biochemical analysis of nutritional and anti-nutritional factors. Seed from distinct plants within accessions was separated, tagged and dried for further research.

SESSION III - Molecular Breeding		
Molecular Detection and Breeding of Tomato Yellow Leaf Curl Virus	LI CHANGBAO	P-III-1
Linkage disequilibrium analysis in a collection of Inra potato breeding lines – First results	Florence Esnault	P-III-2
Use of in silico SSR defined on the reference potato genome sequence to improve a tetraploid genetic map	Marhadour S.	P-III-3
Development of tomato lines for resistance towards *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) in tomato	Mas Muniroh Mohd Nadzir	P-III-4
Candidate gene analysis of carotenoids Synthesis in *Capsicum*	Ayoung Jung	P-III-5
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P-III-1

Molecular Detection and Breeding of Tomato Yellow Leaf Curl Virus

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Tomato yellow leaf curl virus (TYLCV) is currently considered as one of the most devastating viruses in cultivated tomatoes (Solanum lycopersicum) worldwide. We reported here the development of a PCR-based method to quickly detect TYLCV using the primer pairs (TYLCV-F: 5-ACG CAT GCC TCT AAT CCA GTG TA-3 and TYLCV-R: 5-CCA ATA AGG CGT AAG CGT GTA GAC-3), which was designed based on the genome sequence of TYLCV. A TYLCV-specific band of 543 bp was amplified from infected tomato plants. This protocol provides a rapid, reliable, and sensitive tool for molecular detection and identification of TYLCV in the industrial seedling and virus resistance breeding to facilitate safe and sustainable production of tomato.

P-III-2

Linkage disequilibrium analysis in a collection of Inra potato breeding lines – First results

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The development of a genomic selection strategy requires to investigate linkage disequilibrium (LD) pattern along the genome of the species of interest. Potato is a very important staple crop which presents a complex tetrasomic inheritance. Previous studies showed that LD extent in potato depends on the analyzed genetic material and on the genomic regions (D'hoop *et al*., 2010; Stich *et al*., 2013).
This work aims at investigating LD pattern in potato by analyzing a collection of 288 Inra breeding lines that are potentially used by the French breeders in their breeding programs. These breeding lines are maintained in the BrACySol Biological Resource Center, and are originating from different research programs (mainly resistance to late blight, to *Globodera pallida*, to *Pectobacterium*). They were genotyped with the 8303 SNP SolCAP array (Hamilton *et al.*, 2011).
For each SNP, one of the five possible genotypes (AAAA, AAAB, AABB, ABBB or BBBB) was assigned to the breeding lines. To include allelic dosage information in LD characterization, LD was assessed by the calculation of Spearman correlation coefficients. The p-values obtained were corrected for multiple testing using the False Discovery Rate method (Storey, 2002). This poster presents the LD heatmaps that were built for each linkage group.

P-III-3

Use of in silico SSR defined on the reference potato genome sequence to improve a tetraploid genetic map

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The reference potato sequence is a useful resource to develop new markers in regions of interest. Using genetic mapping, we identified, at the tetraploid level, a region of chromosome IX responsible for a significant variation of late blight resistance. Our aim was to get codominant SSR markers able to improve the accuracy of the maps.
A 7,1 Mbp region of the V4.03 reference genome had been determined using Blast of primers already genetically mapped in a ~25 cM interval. 4838 primers pairs were designed with Primer3 around the 5940 microsatellite motifs detected in the region using a local tool. They were tested by *In Silico* PCR, based on Blast algorithm, on the whole genome sequence: 3434 seemed to be unilocus. 48 primers pairs were selected using the following criteria: 4bp repeat length, ~150kbp between the markers, molecular weight between 200 and 400bp. The informativeness of the primers was checked using both parents and 8 individuals of three full-sib families. PCR products were separated using an ABI PRISM® 3100 Genetic Analyzer.
43/48 markers amplified 1 to 5 alleles. 43 to 50 alleles were polymorphic in each family, among them, 15 alleles were common to all. 6 of them originated from one of the late blight resistant parents. We observed an average difference of 20bp (max 80bp) between the molecular weight obtained in our *S. tuberosum* material and the *S. phureja* reference genome. 13 markers were chosen on the basis of the origin of the polymorphism, the number of alleles revealed and their intensity. Mapping on the entire families is underway.

P-III-4

Development of tomato lines for resistance towards *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) in tomato

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The plant pathogen *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) which causes wilting in tomato is considered the most harmful bacterium affecting tomato. The damage caused by this bacterium leads to considerable yield loss of tomato worldwide, and it is considered as a quarantine organism in Europe and many other countries. Many methods have been used to prevent the spreading of the bacteria in the greenhouse and field. One of the more effective ways of combating the disease is through resistance breeding. There are several accessions of wild relatives of tomato known with no or reduced visible symptoms, but they still contain considerable numbers of bacteria. Three quantitative trait loci (QTLs) for resistance mapped on chromosome 5, 7 and 9 originating from *S. arcanum* LA2157 were reported. In our study, the population was further backcrossed to develop near isogenic lines (NILs) and combiNILs to see the effect of single QTL and combination of two QTLs. Disease test, scoring and quantification of bacteria were done to see the level of resistance in these different lines. Future experiments include the level of seed transmission in plants with higher levels of resistance and the spread of bacteria in resistant and susceptible plants.

P-III-5

Candidate gene analysis of carotenoids Synthesis in *Capsicum*

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Carotenoids are vital pigments responsible for yellow, orange and red color in plants. In Capsicum, capsanthin-capsorubin synthase (*CCS*), phytoene synthase (*PSY*), β-Carotene hydroxylase (*CRTZ-2*) and lycopene β-cyclase (*LCYB*) were identified to be involved in the carotenoids synthesis pathway. Previously molecular markers based on the *CCS* and *PSY* genes have been developed to distinguish fruit colors in pepper. However, these markers can distinguish fruit colors of limited pepper genotypes. Therefore, there is need of developing additional markers for accurate prediction of fruit colors using molecular markers. In this study the *CCS, PSY, CRTZ-2,* and *LCYB* genes of 134 pepper accessions were sequenced to identify the genes affecting the fruit color. Sequencing was performed using single molecule real time (SMRT) sequencing technology. We performed two step PCR experiment to reduce sequencing cost. With barcode sequence of primers in the second PCR, we were able to sequence all the samples at one time and detected sequence of each samples. We will develop markers using sequences of in the *CCS, PSY, CRTZ-2*, and *LCYB* genes and these markers will be used for detecting the fruit colors of pepper.

P-III-6

Use of a self-compatible diploid potato for mutagenesis and forward genetic studies

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Potato (*Solanum tuberosum* L.) is one of the major food crops and is cultivated for its starch and nutrient rich tubers, which form on underground stems (stolons). Turbers are very important due to their central role in global food security. However, little is known about the mechanisms underlying tuber formation and regarding how certain architectural plant characteristics can influence the productivity of this crop. The knowledge of the genes controlling important traits such as those involved in crop productivity and quality as well as resistance to biotic and abiotic stresses is being studied through the development of new genomic tools. The publication of the potato genome has provided the physical location of more than 39,000 genes, however there is still a lack of tools for investigating their functions other than the use of transgenic plants or the direct observation of altered phenotypes. The use of high-quality genetic mutant reference collections has shown to be an alternative to genetic transformation techniques for the characterization of important traits in other crops.

We have developed an EMS mutagenized population using an inbred diploid, inter-fertile, resistant to late blight and self-compatible species *S. verrucosum* that can be used to dissect developmental and other traits which may impact directly (e.g. tuber traits) or indirectly on crop yield and quality. To this aim a draft genome sequence of the wild type *S. verrucosum* genotype has been produced. The screening of the F_2 mutant population will provide us mutants carrying interesting traits that are physically located by sequencing and mapping them on the wild type genome.

P-III-7

Initiating Genomic Selection in Potato

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Breeding for more space and resource efficient crops is important to feed the world's increasing population. Potatoes and other crops with storage organs in the soil produce approximately twice the amount of calories per hectare with similar or less input of nutrients and water compared to cereals. The traditional "mate and phenotype" breeding approach is costly and time-consuming; however the completion of the genome sequence of potato has enabled the application of molecular breeding technologies. Genomic selection using genome-wide molecular markers is becoming increasingly applicable to crops as the genotyping costs continue to reduce and is an attractive breeding alternative.

We have used genotyping-by-sequencing to genotype 768 individuals. The individuals were randomly selected from a population of 5,000 individuals derived from a poly-parental cross generated from 18 tetraploid cultivars and breeding clones (MASPot population). Phenotypic data have been established for six agronomical important traits for the entire population.

We have generated statistical models for genomic prediction and have obtained a surprisingly high predictive power with accuracies of 79%, 66%, 78%, and 111% for starch content, late blight resistance, yield, and chipping quality, respectively, when corrected for estimated narrow sense heritability. We expect, however, that the within-population predictive power is considerable higher than out-of population, and we are currently testing an out-of-population panel. Nonetheless, our results suggest that selection of breeding material by genomic selection can be obtained with good prediction accuracies in tetraploid potato.

P-III-8

Linkage disequilibrium and genome-wide association analysis for key breeding traits in eggplant

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The genome-wide association (GWA) approach represents an alternative to biparental linkage mapping for determining the genetic basis of trait variation. The major advantages of GWA lie in being able to sample a much wider range of the phenotypic and genotypic variation present, in exploiting multiple rounds of historical recombination in many different lineages and in including multiple accessions of direct relevance to crop improvement. An eggplant association panel of 191 accessions, comprising a mixture of breeding lines, old varieties and landrace selections originating from Asia and the Mediterranean Basin was SNP genotyped and phenotyped for key breeding fruit and plant traits at two locations over two years. The panel formed two major clusters, reflecting geographical provenance and fruit type. The global level of *linkage disequilibrium* was 3.4 cM. The GWA analysis was performed using the mixed linear model, which takes into account both a kinship matrix and the sub-population membership of the accessions. Overall, 194 phenotype/genotype associations were uncovered, relating to 30 of the 33 measured traits. These associations involved 79 SNP loci mapping to 39 distinct chromosomal regions distributed over all eggplant chromosomes. A comparison of the map positions of these SNPs with those of loci derived from conventional linkage mapping showed that GWA analysis both validated many of the known controlling loci and detected a large number of new marker/trait associations. By exploiting established syntenic relationships between eggplant chromosomes and those of tomato/pepper, orthologous regions in ten eggplant chromosomes were recognized harbouring genes influencing key agronomical traits.

P-III-9

Intronless tandem duplicated class I sHsp genes involved in *Solanum lycopersicum* (cv Heinz 1706) fruit ripening

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The tomato *Solanum lycopersicum* (cv Heinz 1706) is a centerpiece of the Solanaceae family and its genome constitutes a reference in the fleshy fruit development study [1]. Therefore, comparing Solanaceae genomic data is possible to detect gene duplication events and hypothesized their mechanisms of origin. In *S . lycopersicum* (cv Heinz 1706) duplication events are the principal source of small heat shock proteins (*s Hsps*) gene family expansion. Goyal *e t al.* identified an intronless subfamily of cytosolic class I *s Hsps* in the chromosome 6 of in *S . lycopersicum* cv. Ohio 8245 [2]. The subfamily it is conformed by three members genes (Solyc06g076540, Solyc06g076560 and Solyc06g076570) alias Sl20.1, Sl117.6 and Sl120.0. The author described 5' UTR stressresponsive elements, probably involved in abiotic and biotic stress response. We found in tomato cv Heinz 1706 a fourth intronless gene member, Solyc06g076520 that conserve a 97.9% nucleotide identity with Solyc06g076560. Its promoter carries similar cis stressresponsive elements (ERE,Auxin responsive, GARE, HSE, MYB recognition site and ABRElike sequence) that may explain fruit developmental and ripening transcript abundance and differential gene expression pattern in RNAseq experiments. A phylogenomic analysis of Solanum species suggests that duplication event in the chromosome 6 region occurred before the split of the ancestor of tomato related species and potato (~7 MYA) [3]. Since then, their collinear arrangement of 5 class I sHsps intronless genes has been maintained maybe by the effect of natural selection. Nevertheless, in *S . lycopersicum* cultivars the collinear arrangement on the chromosome 6 region might have changed as result of artificial selection.

SESSION V - Flower, Fruit & Tuber Biology		
Fruit Ripening Regulation of α-Mannosidase expression by the MADS Box Transcription Factor RIPENING INHIBITOR and Ethylene	Mohammad Irfan	P-V-1
Suppression of ADP-glucose pyrophosphorylase genes affects fruit skin thickness as well as fruit sugar and sugar phosphate contents in tomato (*Solanum lycopersicum* L.)	Haruka Suzuki	P-V-2
Generation and characterization of ADP-glucose pyrophosphorylase-overexpressing tomato (*Solanum lycopersicum* L.) plant	Hayato Kadowaki	P-V-3
Tomato APETALA3 (TAP3) is involved in fruit set through the regulation of stamen development and GA-Auxin biosynthesis	Yoshihiro Okabe	P-V-4
Impacts of on- and off-vine ripening on tomato fruit hormonal balance	Luciano Freschi	P-V-5
TAGL1/ALQ MADS-box gene participates in the transcriptional regulation of tomato cuticle development	Estela Gimenez	P-V-6
Association of the *bubble fruit* mutant with the process of parthenocarpy in tomato	Hunziker J	P-V-7
Involvement of a new bZip transcription factor in the regulation of tomato fruit development and ripening	M Lemaire-Chamley	P-V-8

P-V-1

Fruit Ripening Regulation of α-Mannosidase expression by the MADS Box Transcription Factor RIPENING INHIBITOR and Ethylene

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α-Mannosidase (α-Man), a fruit ripening-specific N-glycan processing enzyme, is involved in ripening-associated fruit softening process. However, the regulation of fruit-ripening specific expression of *α-Man* is not well understood. We have identified and functionally characterized the promoter of tomato (*Solanum lycopersicum*) *α-Man* to provide molecular insights into its transcriptional regulation during fruit ripening. Fruit ripening-specific activation of the *α-Man* promoter was revealed by analysing promoter driven expression of *beta-glucuronidase* (*GUS*) reporter in transgenic tomato. We found that RIPENING INHIBITOR (RIN), a MADS box family transcription factor acts as positive transcriptional regulator of *α-Man* during fruit ripening. RIN directly bound to the *α-Man* promoter sequence and promoter activation/α-Man expression was compromised in *rin* mutant fruit. Deletion analysis revealed that a promoter fragment (567 bp upstream of translational start site) that contained three CArG boxes (binding sites for RIN) was sufficient to drive *GUS* expression in fruits. In addition, *α-Man* expression was down-regulated in fruits of *Nr* mutant which is impaired in ethylene perception and promoter activation/*α-Man* expression was induced in wild type following treatment with a precursor of ethylene biosynthesis, 1-*aminocyclopropane*-1-carboxylic acid (ACC). Although, *α-Man* expression was induced in *rin* mutant after ACC treatment, the transcript level was less as compared to ACC-treated wild type. Taken together, these results suggest RIN-mediated direct transcriptional regulation of *α-Man* during fruit ripening and ethylene may acts in RIN-dependent and -independent ways to regulate *α-Man* expression.

P-V-2

Suppression of ADP-glucose pyrophosphorylase genes affects fruit skin thickness as well as fruit sugar and sugar phosphate contents in tomato (*Solanum lycopersicum* L.)

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ADP-glucose pyrophosphorylase (AGPase) is a key regulatory enzyme in starch biosynthesis in plant. In tomato fruit, starch accmulattion at early developing stage is important for sugar content in red-ripe stage. We reported two genes encoding ADP-glucose pyrophosphorylase (AGPase), *AgpS1* and *AgpL1* are involved in the starch accumulation in fruit. However, the physiological function of starch and AGPase have not been well investigated in tomato to date. With this aim, in the present study, we generated RNAi transgenic tomato lines with suppressed expression of the *AgpS1* and *AgpL1* genes, and investigated metabolic alterations in developing fruits. Detailed metabolic characterization in a starch deficient line, *35S::AgpS1*^{RNAi} no. 67, revealed that soluble sugars and glucose-1-phosphate contents were respectively decreased by 19-27% and 19-22% in the transgenic compared to the wild-type at the red-ripe stage. Additionally, fruit malate content increased by about 30% in the RNAi lines compared to the wild-type fruit at immature-green and ripening stages, when the respiratory activity increases. Those results indicate i) that the contribution of starch to the fruit sugar content is about 30%, and ii) that glucan phosphorylase is involved in the starch degradation process, which occurs at early ripening in the fruit. Furthermore, the increase in malate, which was observed in the transgenic fruit suggests that there is a trade-off between starch and malate in developing fruits. Interestingly, the starch deficient lines exhibited reduced fruit skin thickness and hemicellulose content at red ripe stage. Those results indicate multiple roles of starch degradative product in tomato plant.

P-V-3

Generation and characterization of ADP-glucose pyrophosphorylase-overexpressing tomato (*Solanum lycopersicum* L.) plant

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Starch accumulation in early developing fruit affects soluble sugar contents in red ripe fruit in tomato. ADP- glucose pyrophosphorylase (AGPase) is a first key regulatory enzyme in starch biosynthesis and is a heterotetramer that consist of two large and two small subunits. In plants, the large subunit functions as allosteric modulator, whereas the small subunit as a catalytic molecule. It has been known that among AGPase-encoding genes, *AgpS1* and *AgpL1* predominantly express in developing fruit in tomato. Our previous work demonstrated suppression of *AgpS1* by RNA infererence resulted in defect of starch accumulation in immature-green fruit and reduced sugar content in red ripe fruits (see the poster presentation by Suzuki et al.). To evaluate the effect of excessive AGPase, in this research, we generated transgenic tomato lines overexpressing either *AgpS1* or *AgpL1* driven by CaMV35S constitutive promoter, and screened 3 and 6 homozygous lines harbouring single transgene in each *35S::AgpS1*^{OX} and *35S::AgpL1*^{OX} at T_0 and T_1 generations. Preliminary characterization revealed fruit Brix (%) value corresponds to the expression level of the transgene in most of the transgenic lines.

P-V-4

Tomato APETALA3 (*TAP3*) is involved in fruit set through the regulation of stamen development and GA-Auxin biosynthesis

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Parthenocarpy is the formation of seedless fruit in the absence of pollination and fertilization. A number of parthenocarpic (*pat*) mutants have been isolated in tomato, and some *pat* loci are likely linked to aberrant stamen development. While several floral homeotic mutations in MADS-box genes are known to cause parthenocarpic fruit development, its mechanism is not well understood. In order to understand the molecular mechanism underlying parthenocarpic fruit development caused by aberrant stamen development, we performed functional analysis of *Tomato APETALA3* (*TAP3/SlAP3*) gene by mutant analysis and RNAi strategy using fruit/ovary specific promoters. Parthenocarpic phenotypes were correlated with severity of stamen abnormality. Increased cell expansion was observed in the mesocarp of *tap3* mutant and *TAP3*-RNAi lines during early-fruit growth. Also, the degree of parthenocarpic efficiency in *TAP3*-RNAi lines was dramatically increased compared to *tap3* mutants, which was accompanied by elevated expression of GA biosynthesis genes including *SlCPS*, *SlGA20ox1*, *SlGA20ox2*, and *SlGA20ox3*, IAA biosynthesis gene *ToFZY*, and auxin signaling gene *SlARF8*, as well as reduced expression of GA-inactivating genes *SlGA2ox1* and *SlGA2ox2* and auxin signaling gene *SlARF7*. These results suggest that *TAP3* is a negative regulator of fruit set through negative regulation of auxin and gibberellin biosynthesis. This work was supported by Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry, Japan (grant no. 26013A)

P-V-5

Impacts of on- and off-vine ripening on tomato fruit hormonal balance

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Ethylene is the master controller of climacteric fruit ripening; however, accumulating evidence also indicates significant influence of other plant hormones on different facets of fleshy fruit ripening. Although fruit tissues apparently possess all the machinery required for hormonal production, these signaling substances and/or its precursors can also be imported from other organs as long as the fruit remains connected to the plant. In this study, the influence of on- or off-vine ripening on tomato fruit hormonal balance was analyzed by using transgenic Micro-Tom plants carrying auxin-, cytokinin- or ethylene-responsive promoters fused to reporter GUS. Hormonal measurements were also performed in fruits from wild-type plants and compared to the activation of the distinct hormone-responsive promoters. Regardless of the ripening conditions (on- or off-vine), the initiation of ripening was marked by a progressive reduction in auxin and cytokinin signaling outputs, which coincided with the climacteric peak of ethylene production. Very low and relatively constant activation of auxin- and cytokinin-responsive promoters was detected at later ripening stages. Auxin and cytokinin signaling output values were several times higher in tomato fruits ripened on vine. These data indicate that the temporal pattern of auxin, cytokinin and ethylene fluctuations in ripening fruits is fairly similar regardless of the ripening conditions; however, the content and signaling output of auxins and cytokinins are considerably higher in tomato fruits ripened on vine, thereby suggesting that significant transport of these hormones from other plant regions to the fruits may take place during earlier ripening stages. (Supported by FAPESP)

P-V-6

TAGL1/ALQ MADS-box gene participates in the transcriptional regulation of tomato cuticle development

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Fruit development and ripening entail essential biological processes, such as cell division and expansion, chlorophyll degradation and carotene synthesis, acid and glucose production, as well as cell wall degradation, which guarantee the seed dispersal and determine productivity and fruit quality traits. Cuticle development has been recently considered as an integral part of the fruit ripening program, whose completion depends in part on composition and biomechanical properties of cuticle. In agreement, fruits silencing *TOMATO AGAMOUS LIKE 1* (*TAGL1*)*/ARLEQUÍN* (*ALQ*), a MADS-box regulatory gene mainly involved in fruit development and ripening, showed significant changes affecting cuticle formation in addition to altered ripening program. *TAGL1/ALQ* repressing fruits displayed a reduction of cuticle thickness and a significant decrease in the content of cuticle components, alterations that made fruit cuticles less resistant than wild-type ones. Accordingly, higher thickness and increased cuticle compound levels were observed in cuticles overexpressing *TAGL1/ALQ*. These biochemical and biomechanical alterations observed in cuticles isolated from silencing and overexpressing *TAGL1* fruits agreed with expression changes of genes involved in cuticle biosynthesis. Furthermore, morphology and arrangement of fruit epidermal cell layers, whose activity largely influences the cuticle formation, were altered when *TAGL1/ALQ* is either silenced or constitutively expressed. Together, these results indicate that *TAGL1*/*ALQ* participates in the transcriptional control of cuticle development likely through the biosynthesis of cuticle components, which in turn would be influenced by epidermal cell patterning. Results also support cuticle development as an integrated event in the fruit expansion and ripening processes which characterize fleshy-fruited species as tomato.

P-V-7

Association of the *bubble fruit* mutant with the process of parthenocarpy in tomato

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Tomato (*Solanum lycopersicum L.*) is one of the most popular fruits produces in the world. Tomato contains lots of nutrients that have benefit for human health and body like antioxidants or vitamins (Passam et all., 2007). Very sensitive to the weather change, we need to perform new cultivars to maintain and increase the yield of production. Several strategies can be used to force fruit production like manual pollination or spray of phytohormone to induce fruit formation by parthenocarpy. Parthenocarpy is development of fruits without pollination. An EMS mutant, *bubble fruit* (*buf*), which produces parthenocarpy, was discovered. The principal phenotype associated to this parthenocarpy is the formation of bubbles at the apical part of the fruit, as the *clausa* mutant (*AVIVI* et al., 2000). The *buf* mutant presents an abnormal ovary development with the development of a second style inside the first one and additional locules. Some primary analysis about hormone content shows an increase in cytokinine and jasmonic acid in bubble part and a decrease of the content of auxin and gibberellin, compare to the corresponding part of WT fruit. It was also find that the ploidy in fruit part increase in the mutant compare to the WT and in opposite, decrease in the bubble part and fruit size compare to the bottom part of WT. Finally, an application of exogenous cytokin induce an increase of the size of the cambium and reduce the bubble phenotype. The application of an antagonist of the GA induces also the reduction of the phenotype and fruit size.

P-V-8

Involvement of a new bZip transcription factor in the regulation of tomato fruit development and ripening

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Tomato (*Solanum lycopersicum*) fruit development is a complex process due to a precise succession of early developmental phases (fruit set, cell division and cell expansion phase) followed by ripening. The completion of fruit development is dependent on multiple regulation processes which are necessary to synchronize fruit development. In particular, several evidences indicate that ripening process is dependent on early regulation events.

In a previous work, a correlation network analysis resulting from transcriptomic and metabolomic characterization of tomato fruit tissues revealed several regulatory hubs possibly involved in the transition between the cell expansion stage and the mature stage of the fruit (Mounet et al., 2009). The functional validation of a bZIP transcription factor was initiated through the generation of a chimeric dominant repressor bZIP-SRDX under the control of the fruit specific SlPPC2 promoter (P_{PPC2}*: bZIP-SRDX* lines) in the MicroTom cultivar.

The expression of *bZIP-SRDX* results in the development of pale green immature fruits due to changes in chloroplast numbers and structure. In addition, P_{PPC2}*:bZIP-SRDX* lines presented profound modifications of fruit ripening including delayed and uneven carotenoid accumulation. Detailed results from the developmental and metabolic characterization of P_{PPC2}*: bZIP-SRDX* lines will be presented. Together with the transcriptomic characterization of P_{PPC2}*: bZIP-SRDX* lines, the phenotypes of the fruits from P_{PPC2}*: bZIP-SRDX* lines x *nr, rin* and *nor* ripening mutants crosses demonstrate the crucial role of this bZip in the regulation of tomato fruit ripening.

SESSION VI - Metabolism and Quality		
Characterisation of candidate genes downstream of the master regulator RIN in Tomato *Solanum lycopersicum*	Jack Gillan	P-VI-1
Control of photosynthetic performance and respiration in arbuscular mycorrhiza symbiosis	Jennifer Göing	P-VI-2
Network Analysis of Transcriptome Reveals Novel Regulations of Potato Pigmentation	Kwang-Soo Cho1	P-VI-3
Molecular and biochemical characterization of high-carotenoid potato germplasm	Giovanni Giuliano	P-VI-4
A 612bp Deletion Is Associated to High Tomatine Level and Bitterness in Tomato Ripe Fruit.	Itay Zemach	P-VI-5
Identification of a 2-Oxoglutarate-dependent Dioxygenase Catalyzing Steroid 16-Hydroxylation in Steroidal Glycoalkaloid Biosynthesis of Solanaceae.	Masaharu Mizutani	P-VI-6
Combining MS and proton NMR metabolomic profiling during tomato fruit development to study environment effect on metabolism	Léa Roch	P-VI-7
Assessment of metabolome compartmentation changes during tomato leaf development using non-aqueous fractionation	C. De Jaham	P-VI-8
Relevance of Sucrose accumulation to the impact of vacuolar processing enzyme (SlVPE5) mediated invertase activation	Ning Wang	P-VI-9
Construction of a pipeline for comprehensive annotation of plant metabolites analyzed by liquid chromatography-high-resolution mass spectrometry	Daisuke Shibata	P-VI-10

P-VI-1

Characterisation of candidate genes downstream of the master regulator RIN in Tomato *Solanum lycopersicum*

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Tomato (*Solanum lycopersicum*) is one of the most extensively consumed fruit crops worldwide. Therefore the identification of genes central to fruit development and ripening, which influence both the quality and nutritional content of the fruit, remains an important objective. Previous work revealed that RIN (ripening-inhibitor) is a master regulator for normal fruit ripening; influencing fruit softening, carotenoid accumulation and aroma formation. The *rin* mutation significantly delayed fruit softening, potentially extending the shelf life of the fruits; however it negatively impacted many other important quality traits. Therefore, downstream targets of RIN have been identified for a more targeted approach for tomato improvement using; Systems Biology outputs derived from transcriptomics performed over fruit ripening and development. Three transcription factors downstream of RIN were identified and knocked out using constructs under constitutive control. Combined phenotypic, metabolite and expression analysis aims to characterise the function of these candidate genes. The results indicate that the transcription factors are important to normal fruit ripening; as the constructs have similar affects to the fruit of the RIN mutants by altering the pigment content and reducing the rate of softening. However, the constructs improve other commercially important quality traits by increasing the rapidity of ripening whilst showing the potential to increase total fruit yield. The project aims to elucidate how these transcription factors influence fruit ripening, and how further manipulation can create varieties with improved physiological and quality traits.

P-VI-2

Control of photosynthetic performance and respiration in arbuscular mycorrhiza symbiosis

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The symbiosis between plant and arbuscular mycorrhiza (AM) is the most common symbiosis of land plants (Glassop *et al.* 2005). AM deliver mineral nutrients and water to the plants and in return obtain carbohydrates from their partner. Zouari *et al.* (2014) showed that the consequences of the association between tomato roots (*Solanum lycopersicum* cv. Moneymaker) and the symbiont *Glomus intraradices* are changes in physiology and primary metabolism of the fruits. However, many details of the involved processes in the leaf are still unclear.
The influence of AM on metabolism of the second fully developed leaf was analyzed. Four weeks post inoculation the experiment started with quantification of photosynthetic capacity by gas exchange measurements. We compared mycorrhizal and non mycorrhizal plant under phosphorus depletion. Growth parameter and shoot phosphorus concentration were similar for both treatments. Gas exchange measurements showed increases in assimilation and transpiration in mycorrhizal plants compared to non-mycorrhizal plants under phosphorus limitation. By gas chromatography-mass spectrometry (GC-MS) analyses we found alterations in metabolites of primary metabolism. Leaves showed differences in glucose, fructose and sucrose content. Additionally, the abundance of metabolites of the TCA cycle as well as amino acids were alter in leaves of mycorrhizal plants compared to non-mycorrhizal plants. The metabolite profile of roots showed variation in sugars, especially trehalose and some amino acids like arginine, cysteine and leucine. The results indicated changes in leaf and root metabolism by AM. The next step in our setup is the analysis of the leaf transcriptome with the aim to identify genes that are up- or downregulated in AM symbiosis.

P-VI-3

Network Analysis of Transcriptome Reveals Novel Regulations of Potato Pigmentation

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Anthocyanins, as a group of flavonoid compounds, are natural compounds for pigmentation of blue-, pink- and red-colored fruits, vegetables and flowers having anti-inflammatory, anti-microbial and anticancer activities and prevention of cardiovascular disease and diabetes in human health. Understanding the regulatory networks that determine the chemical features of anthocyanin may lead more efficient breeding efforts to generate novel color and new patterns of anthocyanins beyond the limits of natural variation. To get insights into the regulatory networks related to anthocyanin biosynthesis and identify key regulatory genes involved in anthocyanin biosynthesis, we performed integrated analysis of transcriptome in sprouting buds germinated from three different colored potato cultivars, light-red Hongyoung, dark-purple Jayoung and white Atlantic. We observed the transcriptional changes using statistical analysis and gene-metabolite correlation networks. Transcript profiles were performed through high-throughput RNA-seq data analysis. Correlation test of anthocyanin contents and transcriptional changes showed 858 strong correlations (correlation coefficient, R^2 > 0.9) between 23 compounds and 120 transcripts categorized to flavonoid metabolism, hormone, transcription regulation and signaling. Their connection network of anthocyanins and genes showed a regulatory system for pigmentation of light-red Hongyoung and dark-purple Jayoung potatoes, suggesting that this systemic approach is powerful to investigate novel genes that are potential targets for the breeding of new valuable potato cultivars.

P-VI-4

Molecular and biochemical characterization of high-carotenoid potato germplasm

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After wheat and rice, potato is the third most important staple food worldwide, largely due to its environmental adaptability, yield potential, and nutritional value. Potatoes are an important nutritional source of starch, vitamin C, protein, and potassium, but a poor source of provitamin A carotenoids. Tuber carotenoids comprise the xanthophylls lutein and zeaxanthin, which are devoid of provitamin A activity but may reduce the risk of age-related macular degeneration. High-carotenoid tubers are common in diploid germplasm, but most tetraploid elite cultivars are carotenoid-poor. Novel, yellow-fleshed potato clones have been developed in the last decade at CRA-CIN. After 7 years of field evaluations one of the clones was released as a new variety named ‘Melrose’. ‘Melrose’ is an early maincrop variety, has deep pink skin and deep yellow flesh, carries resistance to potato cyst nematode Ro1 by H1 gene, is suitable for long storage (tubers with long dormancy), and its tubers are resistant to enzymatic discoloration after cooking (ACB). The tetraploid genotypes were subjected to phenotypic, molecular and metabolic characterization alongside with high-carotenoid diploid cultivars.

P-VI-5

A 612bp Deletion Is Associated to High Tomatine Level and Bitterness in Tomato Ripe Fruit.

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Steroidal glycoalkaloids are secondary metabolites found in the Solanaceae family, which play a major role in plant defense against several pathogens and herbivores. These compounds are anti-nutritional for animals to which they cause gastrointestinal and neurological damage. Tomatine is the main steroidal glycoalkaloid molecule found in tomato leaves and green fruits. Tomatine was found to be associated to red fruit bitterness and there are some natural high tomatine varieties, which all follow "normal" (wild-type) ripening steps. In this study we characterized the *Solanum lycopersicum* bitter accession CC3058. The mature red fruit of this variety exhibit high tomatine levels. Bitterness segregation in 120 F2 plants fits a single gene mode of inheritance with a recessive bitter allele behaving in a very similar manner as was described by Rick et al. (1994). Primary genetic mapping, based on the relatively high polymorphism between *S. pimpinellifolium* and *S. lycopersicum* led to the mapping of the trait to the long arm of chromosome 3. Fine mapping of the bitter mutation, based on recombination events, new markers design, and CC3058 genomic sequencing, revealed a 612bp deletion, which starts shortly before of a peptide transporter and ends in the 3' UTR of the next downstream gene. These results shed new light on the regulation of glycoalkaloids biosynthesis during tomato fruit ripening.

P-VI-6

Identification of a 2-Oxoglutarate-dependent Dioxygenase Catalyzing Steroid 16-Hydroxylation in Steroidal Glycoalkaloid Biosynthesis of Solanaceae.

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Potato (Solanum tuberosum) and tomato (S. lycopersicum) are known to contain steroidal glycoalkaloids (SGAs), which are natural toxins and composed of C_{27} steroids with a nitrogen-containing heterocycle and an oligosaccharide at the hydroxy group of the C-3 position. Potato accumulates a-solanine and a-chaconine and tomato contains a-tomatine. These SGAs are biosynthesized from cholesterol by the oxidations at positions C-16, 22, and 26, the transamination at C-26, the cyclization of E-, and F-rings, and the glycosylation at the C-3 hydroxy group. Previously, we identified two cytochrome P450 genes (SGA1, SGA2), which are involved in the oxidation steps of cholesterol at the C-26 and C-22 positions, respectively. In this study, to explore the other genes involved in SGA biosynthesis, we selected a 2-oxoglutarate-dependent dioxygenase gene (16DOX). Potato 16DOX gene is highly expressed in sprouting eyes, and tomato 16DOX gene is highly expressed in flowers and immature fruits. The 16DOX genes form a gene-cluster with the SGA2 gene on the genomes of tomato and potato. The 16DOX-knockdown transgenic plants exhibited the decrease of the endogenous SGA contents. Biochemical characterization of the recombinant 16DOX protein will be presented.

P-VI-7

Combining MS and proton NMR metabolomic profiling during tomato fruit development to study environment effect on metabolism

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The "Fruit Integrative Modelling" Eranet EraSysBio+ project aimed at describing and modelling the influence of abiotic environmental factors on tomato central metabolism during fruit development (Biais et al. 2014, Plant Physiol. 164:1204). In this project, LC-MS and NMR spectrometry have been used to characterise compounds and estimate their levels in fruit pericarp of tomato plants (*Solanum lycopersicum* cv Moneymaker) cultivated in a greenhouse in control and low-light conditions. During four years, fruits at different stages of development, from 8 days post-anthesis to red-ripe stage, were harvested. ^{1}H-NMR profiling of polar extracts allowed to identify and quantify the primary metabolites in fruit pericarp and visualize the difference between control and low-light condition. Non-targeted LC-QTOF-MS profiling of semipolar extracts was realised to search for specific markers of low-light. The combination of these two analytical strategies with robotized analysis of starch and multivariate analyses allowed characterising the compositional differences between control and low-light conditions during the fruit development. Overall, the effect of stage of development on compositional changes was higher than that of year itself higher than that of light condition. The data generated will be used to study the relationships between primary and specialized metabolism, and parameterize metabolic models.

P-VI-8

Assessment of metabolome compartmentation changes during tomato leaf development using non-aqueous fractionation

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Fruits and vegetables are major components of a healthy diet and could help in the prevention of major illness. In this context, tomato fruit is of great interest, as it is one of the most popular and worldwide consumed vegetables and also the most studied fleshy fruit (Klee and Giovannoni, 2011; Beauvoit et al.,2014; Colombié et al., 2015). Leaves play an important role in the development of tomato fruits, producing their carbon source, mainly as sucrose. However, the carbon balance of leaves changes during their development: young leaves need to import sugars for their growth and become progressively mature source leaves. To study changes in metabolism during leaf development, leaves were harvested at 4 different stages (from young leaves to mature leaves) and metabolic profiles including 68 metabolites (sugars, amino acids, nucleotide sugars, P-compounds, organic acids, polyphenols and glycoalkaloid) were performed. Hierarchical clustering analysis allowed us to separate the 4 successive developmental stages, which are characterized by a progressive decrease in secondary metabolites but also in glycolysis intermediates and nucleotides, resulting from a decrease in energy metabolism; whereas amino acids increased, rather reflecting an activation of anabolism. By using a non-aqueous fractionation (NAF) protocol (Krueger et al., 2011), we estimated the subcellular distribution of metabolites and its modification during leaf development. This study revealed a reprogramming of free sugar metabolism (synthesis and accumulation) and the accumulation of sugars and amino acids into the vacuole in the oldest leaves.

P-VI-9

Relevance of Sucrose accumulation to the impact of vacuolar processing enzyme (SlVPE5) mediated invertase activation

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Enhancing the flavor of fruits plays a fundamental role in improving fruit quality that volatile compositions, acid and sugar accumulation are some of the factors impact on acceptability of sensory responses by human beings. Vacuole in plants not only has functions as a cell compartment which stores amino acids, sugars and other metabolites, but also as a lytic organelle where vacuolar proteins are post-translationally processed into mature forms or degradation by the action of vacuolar processing enzyme (VPE). We have characterized *VPE* genes (*SlVPE1-5*) during fruit development in tomato, and discovered the VPE enzyme activity negatively interfered with sugar accumulation in mature fruits. Comparative proteome analysis demonstrated acid invertase was one of the molecular targets of *SlVPE5*, which is a key enzyme in the sucrose hydrolysis. We established a perfect association of enzyme activity between VPE and invertase, however, the enzyme activity of acid invertase was not likely correlated with mRNA levels. We conclude that *SlVPE5* processes acid invertase into the mature form via post-transcriptional regulation, thereby eventually ensuring *sucrose* hydrolysis, although the suppression of *SlVPE5* results in the failure of the invertase maturation properly, as a *key* step in the mechanism for enhancing *sucrose* content.

P-VI-10

Construction of a pipeline for comprehensive annotation of plant metabolites analyzed by liquid chromatography-high-resolution mass spectrometry

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Various key technologies for analyzing metabolomes have been developed. However, cataloging metabolites from plant species is still a challenging research area. We have been working on computational pipelines to deal huge datasets acquired from mass spectrometry, especially liquid chromatography-high-resolution mass spectrometry (LC-high-resolution MS). In this study we constructed a pipeline using parsley as a model case, which is applicable to other crops including tomato, soybean, lettuce and strawberry. Parsley was grown hydroponically with the medium in which all nitrogen and sulfur atoms in the ingredients were substituted with the stable isotopes ^{15}N and ^{34}S, respectively. Metabolites of grown parsley were extracted and subjected to the analysis by LC-ICR/FT MS, from which all detected chromatogram data were retrieved for the calculation of metabolite peak assignment using our software tool PowerGet. To curate metabolites based on MSn data, we developed a viewer to monitor the scan events, by which two chromatograms, for example, ones from plants grown with or without the stable isotopes are compared at any appointed region of the 2-D imaged chromatograms with the same accuracy. Using the pipeline we annotated 3198 metabolites of parsley in a week. The pipeline we developed will contribute to rapid annotation of crop metabolites, leading to catalogue a whole set of various metabolomes in a formatted manner.

SESSION VII - Systems Biology & Modelling		
Web-Databases PODC and TOMATOMICS for the seamless integration of large-scale omics and knowledge-based information	Kentaro Yano	P-VII-1

P-VII-1

Web-Databases PODC and TOMATOMICS for the seamless integration of large-scale omics and knowledge-based information

Kentaro Yano, Shin Terashima[a], Minami Katayama[a], Tomoyuki Takano[a], Toru Kudo[a], Maasa Kanno[a], Misa Saito[a], Noriko Matsuda-Imai[a], Satomi Asano[a], Koji Yokoyama[a], Koh Aoki[b], Hajime Ohyanagi[a]

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The large-scale omics information markedly accelerate the extension of robust findings in plant science. With the increasing wealth of biological data and knowledge-based information, the new framework in order to effectively and efficiently retrieve accurate and valuable information from big data should be established.
We have developed statistical methodology and GUI software CA_Plot_Viewer to quickly identify genes with similar expression profiles from even large-scale transcriptome data. According to the similarities of gene expression profiles, gene expression networks (GENs) are easily constructed. Our database PODC provides the information on GENs from model crops (http://bioinf.mind.meiji.ac.jp/podc/). Since the GENs among different species are combined with information on orthologues, users can seamlessly access to the information on gene modules, a gene set showing similar nucleotide sequences and expression profiles, among species.
In addition, to expand our understanding of biological functions of gene modules, highly reliable annotations for interactions and functions of genes have been also stored into PODC. The highly reliable annotations have been generated using natural language processing techniques and manual curation of literature. The knowledge-based information is also available from the database TOMATOMICS (http://bioinf.mind.meiji.ac.jp/tomatomics/). The databases will provide more comprehensive omics information obtainable from our bioinformatics infrastructures including systems biology approaches.
This study was partially supported by Computational Software Program from Meiji University.

New Tools for Gene Discovery and Biotechnology		
Bulk segregant QTL analysis of chipping quality in elite potato cultivars	Ea H. Riis Nielsen	P-VIII-1
Effect of cathepsin D inhibitor from *Solanum elaeagnifolium* in an model of wounded *in vitro*	Claudia Lucia Vargas Requena	P-VIII-2
New insights into Solanaceae synteny and identification of conserved loci controlling key agronomic traits in pepper and eggplant	Lorenzo Barchi	P-VIII-3
Functional characterization of IMA/MIF2, a new adaptor peptide involved in floral development	BOLLIER Norbert	P-VIII-4
Toward the rapid identification of causal mutations in tomato EMS mutant populations via mapping-by-sequencing	Cécile Bres	P-VIII-5

P-VIII-1

Bulk segregant QTL analysis of chipping quality in elite potato cultivars

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In recent years genetic markers has been utilized to optimize crops in terms of yield and reducing the use of pesticides and fungicides. Potato production has not kept pace with this development, despite the status as the world's fourth most important crop. In fact, several genetic markers has been suggested by the scientific community, but only few have been embraced by the breeders, as many of the markers has been developed in diploid populations and only few have been tested in an industrially relevant population.
Rapid developments in DNA sequencing and the publication of the Potato Genome is presenting possibilities to 1) mine for candidate genes and genetic variance in elite potato cv near already identified QTL regions and 2) use high throughput DNA sequencing combined with Bulk Segregant Analysis to find novel and known QTL's. Identification of SNP markers linked to these QTL's opens up for the discovery of new and useful genetic markers. In this project we have designed a large industrially relevant population (MASPot) based on 18 elite cultivars and advanced breeding clones used by the Danish breeding company LKF Vandel. The population was phenotyped for chipping quality and two bulks of extreme performers were identified, DNA extracted and sequenced. Following mapping of the sequence data to the genome sequence, we searched for genome regions with differential distribution of genetic variance between the two bulks to identify regions associated with chipping quality. QTL regions were found, of which one overlaps with a QTL from a previous study.

P-VIII-2

Effect of cathepsin D inhibitor from *Solanum elaeagnifolium* in an model of wounded *in vitro*

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The loss of continuity of the skin is accompanied by the activation of cellular mechanisms that accelerate the repair process. Cathepsin D (CD) is an enzyme capable of remove cells and proteins of the extracellular matrix proteins that have been destroyed during injury. The expression of CD is enhanced in acute and chronic injuries; and can avoid or delaying skin repair. In this study, we described the effect of cathepsin D inhibitor isolated from the plant *Solanum elaeagnifolium* (CDI-Se) on the repair of an *in vitro* model wounded, using cells from mouse embryonic fibroblast 3T3. We showed that the concentration of 25 ug / mL CDI-Se allows fibroblasts proliferation in a 17.5%. In the test of wounded, the CDI-Se allowed to close total of the wound at 48 h of incubation, whereas control cells reached to close at 96 h. The expression analysis showed that collagen type 1 and TGF-β1 were present at 4 h of incubation. However, in control test the expression was latter, collagen was expressed until 48 h and TGF-β1 at 4 h of incubation, but the relative expression was smaller than those in presence of CDI-Se. In conclusion, the decrease of the proteolytic activity of CD allows migration and cell proliferation, and it induces the collagen biosynthesis.

P-VIII-3

New insights into Solanaceae synteny and identification of conserved loci controlling key agronomic traits in pepper and eggplant

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Tomato, eggplant and pepper belong to the family of Solanaceae, the third most economically important taxon among cultivated plants. The high number of crops species coupled with an high conservation of genome organization make this family a model for comparative genomics. The genome sequences of tomato, eggplant and pepper are at present available, however the fragmentation and limited of anchorage of the eggplant genome sequence limits its use for synteny and comparative studies. The chromosome structural relationships among the three species were investigated by aligning (GMAP software) mapped eggplant RAD-tag (restriction-associated DNA) markers on tomato and pepper genome (CM334) as well as tomato CDS on pepper genome. Results confirmed most of the chromosome rearrangements previously identified, but new ones were also spotted.
Eggplant and pepper orthologous QTL were also identified by aligning both eggplant RAD-tag and pepper SNP markers on pepper genome. A set of 212 eggplant QTL, of which 76 major QTL (PV explained ≥ 10%), as well as 151 pepper QTL, were located on pepper genome sequence.
Overall, fourteen clusters of co-localizing markers were identified, of which 8 comprising eggplant and pepper analogous QTL and 6 including eggplant QTL without the pepper counterpart. Candidate gene analyses will be performed to identify the gene sequences lying on QTL confidence intervals. The availability of the upcoming high quality eggplant genome sequence will improve the resolution of syntenic analyses as well as the identification of new conserved agronomic loci on eggplant.

P-VIII-4

Functional characterization of IMA/MIF2, a new adaptor peptide involved in floral development

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The **MI**ni zinc **F**inger (MIF) gene family encodes small proteins involved in the regulation of floral development and hormonal signaling pathways (Hu and Ma, 2006). Mini zinc Finger proteins only display a non-canonical zinc finger domain and cannot be defined as transcription factors because they do not bind to DNA. The molecular function of MIFs is linked to this unique domain, an unusual zinc finger, which confers to MIF the capacity to interact with other proteins, thus providing their ability to control cellular and physiological processes.
Constitutive over-expression of *MIF2* in *Arabidopsis* or of its ortholog *IMA* (***I**nhibitor of **M**eristem **A**ctivity*) in *Tomato* cause pleiotropic developmental defects such as a bushy phenotype and a dramatic alteration of flower and fruit development (Sicard *et al*., 2008) linked to the capacity of IMA/MIF2 to interact and interfere with target protein function.
Using various molecular tools, we aim at investigating the Mini Zinc finger protein function and at deciphering the associated molecular and regulatory mechanisms.
First, we made use of the Gateway/GoldenBraid assembly method to generate numerous molecular constructs as to test protein-protein interactions, promoter expression with reporter gene and multiple overexpressions.
Second, we used CRISPR/Cas9 approaches, not only to investigate the effects of a loss of function in our genes of interest, but also to test the consequences of deleting important structural domains in the proteins to investigate their importance in the protein function.

P-VIII-5

Toward the rapid identification of causal mutations in tomato EMS mutant populations via mapping-by-sequencing

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Tomato is currently the model plant for fleshy fruit development and for *Solanaceae* species. Recent genomic approaches including transcriptome, proteome and metabolome analyses and genetic mapping have produced a wealth of candidate genes whose function needs to be assessed. Forward genetics appears as the most powerful approach for the identification of new gene functions. Mutant collections offer invaluable resources for discovering new phenotypes and new allelic variants. Thanks to the recent availability of tomato genome sequence and of deep sequencing tools, linking phenotypic changes to the causal genotypic variations is now more accessible.
We generated highly-mutagenized tomato EMS mutant resources for reverse and forward genetic approaches in tomato using the miniature tomato cultivar Micro-Tom. We describe here the use of this mutant collection for rapid discovery of genes underlying remarkable traits in tomato fruit through the identification of causal mutations by mapping-by-sequencing. We also give some indications about the bioinformatics analysis of the mutants regarding the distribution of EMS mutations, the mutation types and the possible effect of mutations on gene function. In summary, the results presented herein provide genome information on Micro-Tom EMS mutants and propose a strategy aimed at the identification of genes responsible for traits of interest in tomato.

SESSIONS IX et X - Biotic stress		
Comparative transcriptome analysis of resistant and susceptible tomato lines in response to infection by *Xanthomonas perforans* race T3	Wencai Yang	P-IX-1
Identification, by RNAi silencing, of susceptibility genes involved in *Phytophthora infestans* infection in potato	Kaile Sun	P-IX-2
Development of a *Orobanche aegyptiaca* parasitism-competent tissue to tomato root in liquid culture	Koh Aoki	P-IX-3
cDNA cloning, identification, tissue localisation, and transcription profile of Cathepsin D inhibitor from *Solanum elaeagnifolium*	Florinda Jiménez Vega	P-IX-4
Improving pathogen resistance in tomato by impairing plant susceptibility genes	Van Tuinen A	P-IX-5
A role for long-distance RNA signaling in tomato via graft-induced vigor	Frank M	P-IX-6

P-IX-1

Comparative transcriptome analysis of resistant and susceptible tomato lines in response to infection by *Xanthomonas perforans* race T3

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Bacterial spot, incited by several *Xanthomonas* sp., is a serious disease in tomato (*Solanum lycopersicum* L.). Although genetics of resistance has been widely investigated, the interactions between the pathogen and tomato plants remain unclear. In this study, tanscriptomes of *X. perforans* race T3 infected tomato lines were compared to those of controls. An average of 7million reads were generated with approximately 21,526 genes mapped in each sample post-inoculation at 6h (6 HPI) and 6d (6 DPI) using RNA-sequencing technology. Overall, the numbers of differentially expressed genes (DEGs) were higher in the resistance tomato line PI 114490 than in the susceptible line OH 88119, and the numbers of DEGs were higher at 6 DPI than at 6 HPI. Fewer genes (78 in PI 114490 and 15 in OH 88119) were up-regulated and most DEGs were down-regulated, suggesting that the inducible defense response against race T3 might not be activated at 6 HPI. Accumulation expression levels of 326 co-up regulated genes in both tomato lines at 6 DPI might be involved in basal defense, while the specific and strongly induced genes at 6 DPI might be correlated with the resistance in PI114490. The dominant subcategories of DEGs in both tomato lines identified by GO functional enrichment were Cellular process, Cell and Catalytic activity. A number of DEGs related to the KEGG pathways of plant hormone signal transduction and plant-pathogen interaction pathway. The results will provide a valuable resource for understanding the interactions between *X. perforans* and tomato plants.

P-IX-2

Identification, by RNAi silencing, of susceptibility genes involved in *Phytophthora infestans* infection in potato

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Multiple susceptibility (S) genes in Arabidopsis involved in the interaction with different pathogens, have been identified. Impairment of these genes results in disease resistance which can be sustainable as observed with the *mlo* gene in barley against powdery mildew. It is unknown whether these genes are conserved in different crop plants, and whether impairment of such genes could provide resistance to *Phytophthora infestans* in potato. In our study, we identified potato orthologs corresponding to about 11 *Arabidopsis* S-genes by mining the potato genome. RNAi silencing of a few of these S-genes in potato resulted in resistance to *P. infestans*. It indicates that these S-genes are required for successful infection of potato by *P. infestans* and that disabling of these genes leads to disease resistance in potato.

P-IX-3

Development of *Orobanche aegyptiaca* parasitism-competent tissue to tomato root in liquid culture

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Root parasitic plant, *Orobanche aegyptiaca*, obtains water and nutrient from host plant. Although *Orobanche aegyptiaca* is a holoparasitic plant, it grows in an in vitro culture condition in the absence of a host plant. Callus grown in the in vitro culture has a capability to form tubercle on the tomato (*Solanum lycopersicum*) root. However, the developmental process of *O. aegyptiaca* callus has not been described so far. It has not been clarified whether the parasitizing process of the callus and that of radicle are identical or not.

In the present study, *O. aegyptiaca* radicles were grown into callus in liquid culture system. We observed developmet of two types of calli, root-like callus and amorphous callus. When fragments from the root-like callus were inoculated to tomato root in vitro, new protrusions developed from the fragment and established parasitic connection with tomato root. Rate of successful in vitro inoculation was as high as radicle generated from seed. We then observed morphology of root-like- and amorphous callus by light microscopy. In the amorphous callus, we found high cell-density region near the tip of the tissue, however, did not found vascular tissue along the longitudinal axis of the tissue. On the other hand, in the root-like callus, we found the presence of the xylem tissue, suggesting that vascular tissue has already differentiate along the longitudinal axis of the root-like tissue. Taken these results together, the root-like callus with differentiated vascular tissue is competent with respect to the parasitism. Developmental relationship between the root-like- and the amorphous callus will be discussed. This work was partly supported by Grant-in-Aid for Scientific Research on Innovative Areas.

P-IX-4

cDNA cloning, identification, tissue localisation, and transcription profile of Cathepsin D inhibitor from *Solanum elaeagnifolium*

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The plants have constitutive and induced defense mechanisms to resist biotic and abiotic attacks that they are exposed. The induced mechanisms can cause changes in cell stability by the presence of secondary molecules. These molecules help to maintain resistance against organisms such as viruses, fungi, bacteria, insects and vertebrate herbivores up. One of the molecules involved in their defense system are protease inhibitors. In this study, the codifying sequence for the cathepsin D inhibitor (CDI) was identified from *Solanum elaeagnifolium*, which is 660 pb length and 99% similar to the homologous reported in other solanaceae as potato and tomato. The conceptual translation produces a 220 aa protein with three inhibitory sites: the first one correspond to cathepsin D (R99), and the others against trypsin and chymotrypsin, respectively. Structurally, this molecule presents six conserved cysteins within the STI domain and additionally a Kunitz motif. The protein has a molecular weight of 24.5 kDa and a predicted pI of 8.4. Furthermore, according to the expression analysis, CDI is found mainly in the green fruit and no in mature or yellow fruit. The major expression occurs in fruit (100%), stalk (65%), sheet (56%) and root (22%). This information will contribute to physiological studies of *Solanum*, and the probable biotechnology use of the plant and/or its inhibitors in industrial and therapeutic processes.

P-IX-5

Improving pathogen resistance in tomato by impairing plant susceptibility genes

Van Tuinen A, Bokhoven-Schipper Da, Sun Ka, Wolters AMa, Van Kan Jb and Bai Ya

In tomato (Solanum lycopersicum) diseases such as late blight (Phytophthora infestans), grey mold (Botrytis cinerea) and powdery mildew (Oidium lycopersici) can lead to severe crop losses. Host specific resistances (R-genes) are often overcome by the pathogen and do not lead to a permanent solution. Lately, plant factors which suppress plant immunity upon activation by pathogen effectors have come in view. Disabling of these so called plant disease susceptibility genes (S-genes) could lead to a more durable and broad-spectrum resistance. For Arabidopsis more and more mutants with altered disease sensitivity i.e. candidate susceptibility genes are reported. Whereas a lot of these genes have unwanted pleiotropic effects, milder alleles could have a better balance between enhanced disease resistance and cost of fitness and used as source for a more durable resistance level.
We have developed an EMS population of tomato cultivar Micro-Tom. For the identification of mutants both forward and reverse genetics are used. So far, we have screened 700 of the 4500 M2 families by Detached Leaf Assays (DLA) or whole plant infection for segregation of reduced susceptibility to late blight and grey mold, and powdery mildew, respectively and/or aberrant morphology.
Candidate S-genes were selected and DNA of 2950 M1's has been used for Targeting Induced Local Lesions IN Genomes (TILLING) and deep sequencing. Point mutations causing deleterious effects at the protein level were identified in several pools, which will be re-sequenced for confirmation and identification of the individual M1 plants.

P-IX-6

A role for long-distance RNA signaling in tomato via graft-induced vigor

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Grafting has been used to increase yield, especially in the context of biotic and abiotic stress for over 2000 years. Although recent experimental evidence suggests that non-cell autonomous long-distance signals may play an important role in the mechanism through which grafting impacts plant growth and physiology, the precise identity of these signals and the mechanisms by which they act to affect yield remain largely unexplored. In tomato, the grafting of elite fruit producing shoots (scions) onto vigorous, interspecific hybrid root systems significantly increases yield. Here, we present data showing that grafting-induced vigor can be reciprocally transferred between the root and shoot systems of an interspecific hybrid (*Solanum lycopersicum* x *S. habrochaites*) and domesticated (*S. lycopersicum*) tomatoes. We combine these measurements with RNA-seq profiles from reciprocally grafted root and shoot systems in order to identify non-cell autonomous graft-transmissible transcripts that may serve as molecular signals through which grafting-induced vigor is conferred.

Session XI - Abiotic Stress		
Determination by near infrared microscopy (NIRM) of nitrogen content in tomato (*Solanum lycopersicum* L.) leaf powder	Gauthier Lequeue	P-XI-1
Understanding the genetic basis for salinity tolerance in Galapagos tomatoes	Yveline Pailles	P-XI-2
Ozonated water promotes growth and development of tomato seedlings under low temperature condition?	Kazuhisa Kato	P-XI-3
Tobacco LTR retrotransposon-Gene associations and their expression in response to stress	BUI Quynh Trang	P-XI-4
Role of the MYB33, MYB101 and the MYB65 transcription factors in plant response to water deficiency.	Anna Wyrzykowska	P-XI-5

P-XI-1

Determination by near infrared microscopy (NIRM) of nitrogen content in tomato (*Solanumlycopersicum* L.) leaf powder.

Lequeue Gauthier.[a], Baeten Vincent.[b] and Draye Xavier.[a]

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The chemical analysis of plant samples is time consuming and expensive. Near infrared spectroscopy techniques were developed, *inter alia*, as a rapid technique to predict the chemical composition of foods. In this study near infrared microscopy (NIRM) was used for the first time to determine the nitrogen content in reduced quantity of tomato leaf powder.
A total of 72 tomatoes sample corresponding to 6 groups of 12 plants under 6 levels of nitrogen concentration. This experiment was performed with 3 simultaneous repetitions (216 samples submitted to analysis in total). For the model constructed, 30 samples were used in the calibration stage and 30 samples were used in the independent validation stage. The calibration and validation sets analyzed were chosen to cover the full range of spectra variation obtained from the NIRM analysis. The nitrogen content was determined by combustion according to the Dumas method.
Standard error of calibration (SEC), coefficient of determination at the calibration stage (R^2c) and ratio of performance to deviation (RPDc) were excellent (R^2c values higher than 0.90 and RPDc values higher than 3).
The coefficient of determination at the validation stage (R^2p) and standard error of prediction were excellent (R^2p values higher than 0.90). A model using all the information from the samples of the calibration and validation sets was constructed to improve the accuracy of the future prediction.
The study indicated that NIRM is a promising and suitable tool for a rapid, non-destructive and reliable determination of the chemical composition in tomato leaf powder.

P-XI-2

Understanding the genetic basis for salinity tolerance in Galapagos tomatoes

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Wild relatives of food crops provide valuable genetic resources for tolerance to various abiotic stresses. Of particular interest are two species of tomato endemic to the Galapagos Islands: *Solanum cheesmani*ae and *S. galapagense.* Besides being close relatives of *S. lycopersicum*, they are tolerant to salinity and often grow near the coast, making them suitable for the study of the salinity tolerance in tomatoes. For this purpose, we characterized 69 accessions of Galapagos tomatoes and two varieties of *S. lycopersicum*.
We have sequenced and assembled the draft genomes of the two Galapagos tomato species. In addition, a DArTseq analysis has been performed to genotype all 69 accessions and to establish the population structure of our germplasm collection.
Phenotypic studies at the seedling stage have been performed, subjecting the seedlings to 200mM NaCl for 10 days after leaf emergence. The NaCl was administered gradually and supplemented with $CaCl_2$, to maintain constant Ca^{2+} availability. Various phenotypes were recorded and analysed for their contribution to salinity tolerance, compared to control conditions. Six out of the 69 accessions were selected based on their good performance under salinity. The six accessions are now being studied for their performance under salinity throughout their life cycle, up to the reproductive stage. This experiment includes the scoring of several physiological parameters through time and RNA extraction for transcriptome analyses and exome sequencing.
The expected outcomes are the understanding of the genetic mechanisms that confer salinity tolerance in tomatoes and the development of mapping populations for further genetic studies.

P-XI-3

Ozonated water promotes growth and development of tomato seedlings under low temperature condition?

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Ozone is a strong oxidizing agent, so ozonated water (OW) is used as a germicide in agriculture. On the other hand, there are few information about the effects of OW treatments on plant growth and development, so those effects were investigated in this study. OW was prepared by aerating tap water (TW) with air stone for 15 min. TW were overhead-irrigated every day, and OW was treated once (OW1) or three times (OW3) per week instead of TW during raising seedlings. Two tomato cultivars ('Micro-Tom' and 'Momotarou8') were cultivated in spring 2012. The plant growth and development were promoted by OW3. The OW treatments were also performed in four cultivars ('Micro-Tom', 'Aiko', 'Natsunokoma' and 'Momotarou8') during raising seedlings in spring 2013. OW was treated once, three or seven times per week instead of TW. However the plant growth and development were not promoted by OW treatments. We thought the effect by OW treatment was depend on the environment during cultivation and the temperature might be important. Therefore we tested in the cultivation of Komatsuna (*Brassica rapa* L. Perviridis Group) as a model plant for treatment of OW under low and adequate temperatures in growth chambers. The plant growth and development were affected under only low temperature condition, so it was assumed that the cold-stress tolerance were increased in Komatsuna by OW treatments. Thus we also have to make experiments using tomato in the near future.

P-XI-4

Tobacco LTR retrotransposon-Gene associations and their expression in response to stress

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Retroviral-type LTR elements are major components of plant genomes. They represent promoter/regulatory capsules that can drive the production of chimeric co-transcripts with adjacent genes in response to their own specific expression pattern. These elements can be activated by stress and may be involved in the host response to specific stimuli.

Tobacco transcriptome analysis revealed an abundance of LTR retrotransposon-gene associations and these co-transcripts were over-represented in stress conditions. We have identified many co-transcripts originating from the known tobacco LTRs. These co-transcripts extend into downstream adjacent sequences, including genic sequences. Our experimental analysis confirm that 3'LTRs can drive the synchronous expression of these co-transcripts in conditions where retrotransposons are transcriptionally activated, such as microbial elicitors or wounding. The retrotransposon response to stress and associated co-transcript production varies depending on the element. In parallel, we are extending our analysis to a larger range of retrotransposons reconstructed from tobacco shotgun genomic data, and are developping a 454 RNA-Seq analysis of retrotransposon expression and chimeric co-transcripts production in stress conditions.

Our current hypothesis is that plant retrotransposons may act as intermediates of stress stimuli, redirecting messages towards cellular functions in stress conditions.

P-XI-5

Role of the MYB33, MYB101 and the MYB65 transcription factors in plant response to water deficiency.

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The goal of the project is to explain the role of the MYB33, MYB101 and the MYB65 transcription factors in the *Arabidopsis thaliana* and *Solanum tuberosum* var. Desiree plants response to drought stress. We rise a working hypothesis that among factors responsible for the increased plant tolerance to water deficiency is the increased level of several *MYB* genes expression. In our previous studies we silenced expression of the *CBP80*/*ABH1* gene that is involved in the ABA signaling pathway. In the transgenic lines, with no induction of the microRNA159 expression we observed the increased level of the *MYB33* and the *MYB101* genes expression, of which mRNAs are targets of the miR159-mediated cleavage. The *CBP80/ABH1* - silenced plants revealed the increased tolerance to drought. Also we idnetified null Arabidopsis mutants of *MYB101* and *MYB33* genes and found that those plants are oversensitive to drought conditions in comparison to the wild type plants. This result confirms our hypothesis on the role of the MYB TFs studied in repsonse to water defficiency. In this project we plan to obtain transgenic Arabidopsis and potato lines over-expressing *MYB33, MYB65, and MYB101* genes. The introduced *MYB* TF genes are mutated to be resistant to miR159-guided cleavage. Wild type and mutant plants, cultivated in control and water deficiency conditions will be compared (their phenotypes and selected physiological traits). We expect that our results will help to better understand the connections and relationships between the various genes studied and to determine their impact on water deficiency tolerance in plants.

Parallel Session - Tomato		
Fine mapping and phenotypic characterisation of the *bushy* root mutation	Silva Ferreira D	P-TO-1
The identification of pistil-specific genes by global transcriptome analyses in tomato (*Solanum lycopersicum*).	Ezura K	P-TO-2
Development of Micro-Tom Bioresources: Mutant collection and genome information	Hoshikawa K,	P-TO-3
Understanding hybrid seed failure in wild tomatoes: phenotypic and transcriptomic signatures	Roth M	P-TO-4
Higher yield and more uniform fruit set in selections of the 'Valenciana' local tomato landrace	Soler S	P-TO-5
The glycero-3-phosphate acyl transferase GPAT6-like from tomato plays a crucial role in fruit cutin biosynthesis	Petit J	P-TO-6
Development of Micro-Tom Bioresources: Mutant collection and genome information	Hoshikawa K	P-TO-7
Fruit Ripening Regulation of α-Mannosidase expression by the MADS Box Transcription Factor RIPENING INHIBITOR and Ethylene	Irfan M	P-TO-8
Suppression of ADP-glucose pyrophosphorylase genes affects fruit skin thickness as well as fruit sugar and sugar phosphate contents in tomato (solanum lycopersicum L.)	Suzuki H	P-TO-9
Suppression of Tomato Prolyl 4 Hydroxylase 3 results in alterations on fruit development, ripening and abscission.	Kalaitzis, P	P-TO-10

P-TO-1

Fine mapping and phenotypic characterisation of the *bushy root* mutation.

Demetryus K. S. Ferreira[a], Fady Mohareb[a], Zoltan L. Kevei[a], Lazaro E. P. Peres[b] and Andrew J. Thompson[a]

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The tomato mutant *bushy root* (*brt*) arose from a cross between tomato accessions "Stock No. 1" and cultivar "Red Cherry" following EMS mutagenesis of pollen (Zobel, 1972). Due to the mixed genetic background, and aiming to introduce the mutation into a rapid cycling and dwarf genetic background, *brt* was introgressed into Micro-Tom (MT) via six backcrosses, producing a homozygous near isogenic line (*brt*-NIL). Small seed size was observed in the *brt*-NIL, which seems to be a pleiotropic effect of *brt*. After comparison with MT and reciprocal *brt*-NIL x MT F_1 and F_2 seeds, it was determined that this phenotype is controlled by the maternal tissue. The small seed size is likely to explain the slow early development exhibited by *brt*-NIL. The mutant root system was characterised by image analysis and can be described as compact and "bushy", but this effect is created not by a change in the number of lateral roots per unit length of root, but due to the reduced length of the primary root and each lateral. MT (Kevei et al 2015) and *brt*-NIL genomes were re-sequenced and assembled using the *S. lycopersicum* Heinz 1706 reference genome (version SL2.50). This showed that the introgression spans 64 Mbp of the 67 Mbp of chromosome 12. The target region was then reduced to 0.4 Mbp and a number of recombinant lines have been recovered from a F_2 population. Current progress in fine-mapping the *brt* locus and candidate genes will be presented.

P-TO-2

The identification of pistil-specific genes by global transcriptome analyses in tomato (*Solanum lycopersicum*).

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Parthenocarpy, a developmental transition of unfertilized ovary into fruit, is a desirable trait for fruit-bearing crops such as tomato (*Solanum lycopersicum*). Current understanding of its mechanim is that several plant hormone signals are constitutively active in the unfertilized ovary, and mimick pollination-dependent fruit set process. However, little is known about the molecular mechanism regulating parthenocapry.
To further expand our understanding of this process, this research isolated tomato pistil-specific genes through comprehensive RNA-seq based transcriptome analysis using different 17 tissues or developmental pistil stages. First, 532 candidate genes which are specifically/preferentially expressed in pistil were identified based on the tissue expression profiles. Next, we compared our RNA-seq data with public available transcriptome data, refining the candidate genes that are specifically expressed within the pistil.
As a result, we isolated several trascription factors including homologous genes that are important for reproductive development such as *CRABS CLAW YABBY* gene. Furthermore, new peptide hormone-like genes were isolated; cyteine-rich peptide like gene that has a secreted signal and conserved cysteine residues in some *Solanaceae* species. It has been revealed peptide hormones function as signaling molecules in various process of plant development and growth. These genes may play a specialized role in the regulation of pistil development related to fruit set.

P-TO-3

Development of Micro-Tom Bioresources: Mutant collection and genome information

Hoshikawa K[a], Ariizumi T[a], Fukuda N[a], Kanayama Y[b], Kubo Y[c], Aoki K[d], and Ezura H[a]

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Tomato is an excellent model plant for fruit biology research and for genomic studies of the *Solanaceae* family including eggplant, pepper, petunia and tobacco. To facilitate breeding and functional genomics research of tomato, we launched on the tomato bioresource program in 2007 within the framework of the National BioResource Project (NBRP) in Japan (NBRP tomato; http://tomato.nbrp.jp/). We chose cultivar Micro-Tom as a model system that has advantages as a model plant including small plant size, a short life cycle and the availability of functional genomics tools. As mutant resources in NBRP tomato, we have produced >13,000 mutant lines by ethylmethanesulfonate (EMS) treatment and gamma-ray irradiation and have isolated 1,890 individual mutants with visible phenotype from the mutant population so far. The visible phenotype data have been uploaded to a tomato mutant database 'TOMATOMA' (http://tomatoma.nbrp.jp/), and these mutant seeds are available via this database. To provide important and valuable information for fruit research, we are also accumulating metabolic profiles of the tomato mutants, including amino acid composition, carotenoid contents and Brix values. In addition to the Micro-Tom mutants, we started to provide T-DNA tag lines of Micro-Tom recently. On the other hand, as DNA resources, the sequence information of Micro-Tom full-length cDNA and EST is available from database 'KaFTom (http://www.pgb.kazusa.or.jp/kaftom/) and EST database 'MiBASE' (http://www.pgb.kazusa.or.jp/mibase/), respectively. A reference genome sequence of Micro-Tom will be available through 'TOMATOMICS' (http://bioinf.mind.meiji.ac.jp/tomatomics/). These tomato resources will accelerate research and development of tomato and fleshy fruits.

P-TO-4

Understanding hybrid seed failure in wild tomatoes: phenotypic and transcriptomic signatures

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Hybrid seed failure is a common reproductive barrier between plant species. For plant breeders, it represents a major obstacle to introgression of desirable traits from wild to domesticated species. Postzygotic barriers to hybridization have been well-documented among wild tomato species, and histological work showed that endosperm failure is the main cause of seed abortion. Based on an updated phylogeny of the tomato clade, we addressed hybrid seed failure between three taxa, namely *Solanum peruvianum*, *S. chilense* and *S. arcanum* var. maranon). We characterized mature seed size and viability in a large number of crosses and conducted endosperm-specific RNAseq experiments using six reciprocal crosses within and between the three taxa. The crossing design was chosen to detect expression pattern differences with regard to overall and parent-of-origin- specific expression (i.e. imprinting). Reciprocal interspecific crosses involving *S. peruvianum* yielded no viable seeds and were classified as having a 'strong' barrier. Crosses between *S. chilense* and *S. arcanum* var. maranon were characterized by variable levels of seed viability as well as asymmetric outcomes in some reciprocal crosses, here classified as a 'soft' barrier. Seed size was significantly reduced with *S. peruvianum* in the maternal role in hybrid crosses, compared to crosses within this species. Transcriptome analyses shows drastic expression changes when comparing intraspecific crosses –with normally developing endosperm– and among-species crosses with abnormal endosperm. We propose imprinting disturbance as a mechanism contributing to hybrid seed failure, but ongoing work will further characterize molecular pathways possibly involved in this reproductive barrier and its variability.

P-TO-5

Higher yield and more uniform fruit set in selections of the 'Valenciana' local tomato landrace

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The local tomato landrace 'Valenciana', which is characterized by having large heart-shaped fruits with firm and meaty flesh and very few seeds, is very popular in its home region of València (Spain). 'Valenciana' tomato is produced from the end of spring to beginning of autumn reaching much higher prices than the extensively cultivated long shelf-life tomato varieties. Morphological characterization studies carried in the framework of the TRADITOM project have revealed that there is diversity for agronomic traits within this landrace. We have evaluated the distribution of a series of yield-related parameters in both the original non-selected 'Valenciana' plant material and in three selections derived from it that were obtained by our group (211, 767, and 886). An F1 hybrid (Eufrates) which is frequently grown in the region was used as a reference of improved commercial variety with large and long shelf-life fruits. Although total fruit yield was significantly higher in Eufrates (6.9 kg/plant) than in the 'Valenciana' materials, selections 211 and 767 (5.2 and 5.0 kg/plant, respectively) had a significantly higher yield than selection 886 and the non-selected landrace (4.4 and 4.3 kg/plant, respectively). The number of flowers per inflorescence was very uniform in Eufrates, with an average of 6 flowers/truss, while in the 'Valenciana' materials the number of flowers was much higher in the three first trusses (between 10 and 14 flowers per truss) and decreased in the upper (6^{th}-7^{th}) trusses to 6-8 flowers per truss. Regarding the number of fruits set per truss, Eufrates presented a quite regular fruit set, ranging from around 5 fruits in the first truss to around 3 fruits in the 7^{th} truss, while in 'Valenciana' materials the number of fruit set decreased progressively from the first truss (between 5 and 8 fruits per truss) to the 7^{th} truss, in which very few fruits (between 0 and 0.5 on average) set. Selections 211 and 767 had a similar or greater fruit set in all trusses than selection 868 and the non-selected landrace. The results indicate that higher yield and fruit set distribution can be selected in 'Valenciana' tomato in order to improve its agronomic performance and consequently its value. Selections 211 and 767 will be evaluated in extensive field trials and may be eventually registered as improved 'Valenciana' selections. This may contribute to the enhancement of local tomato varieties, which is one of the major objectives of the TRADITOM project.

P-TO-6

The glycero-3-phosphate acyl transferase GPAT6-like from tomato plays a crucial role in fruit cutin biosynthesis

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Tomato mutants recently proved their considerable interest for identifying key players of cuticle formation and for deciphering their role in the fruit. In a recent study, we isolated a tomato fruit glossy mutant (P23F12 line) by screening an EMS mutant collection in the miniature cultivar Micro-Tom [1]. Using a newly developed mapping-by-sequencing strategy, in which BC1F2 bulks segregating for the glossy trait were submitted to whole-genome sequencing, we identified the underlying causal mutation. The point mutation introduces a charged amino acid next to the active site of a GPAT6-like enzyme, whose function is to couple glycerol 3-phosphate with ω-hydroxy fatty acids to produce cutin monomer precursors. The P23F12 glossy mutant was affected in pollen formation but did not show any male sterility, unlike mutations in its Arabidopsis putative ortholog. In addition, both cutin and wax composition were strongly modified in the mutant fruit, thereby leading to strong alterations of the cuticle properties. Transcriptome analysis of mutant fruit exocarp using RNA seq further highlighted the main processes and pathways affected at transcriptional level, among which the lipid, cell wall and secondary metabolite biosynthesis pathways. Thus, besides its crucial role in fruit cutin biosynthesis, the inactivation of the tomato GPAT6-like enzyme likely leads to profound alterations of various aspects of cuticle formation.

P-TO-7

Development of Micro-Tom Bioresources: Mutant collection and genome information

Hoshikawa K[a], Ariizumi T[a], Fukuda N[a], Kanayama Y[b], Kubo Y[c], Aoki K[d], and Ezura H[a]

[a] University of Tsukuba, Tsukuba, 305-8572, Japan; [b] Tohoku University, Sendai, 981-8555, Japan; [c] Okayama University, Okayama, 700-8530, Japan; [d] Osaka Prefecture University, Sakai, 599-8531, Japan

Tomato is an excellent model plant for fruit biology research and for genomic studies of the *Solanaceae* family including eggplant, pepper, petunia and tobacco. To facilitate breeding and functional genomics research of tomato, we launched on the tomato bioresource program in 2007 within the framework of the National BioResource Project (NBRP) in Japan (NBRP tomato; http://tomato.nbrp.jp/). We chose cultivar Micro-Tom as a model system that has advantages as a model plant including small plant size, a short life cycle and the availability of functional genomics tools. As mutant resources in NBRP tomato, we have produced >13,000 mutant lines by ethylmethanesulfonate (EMS) treatment and gamma-ray irradiation and have isolated 1,890 individual mutants with visible phenotype from the mutant population so far. The visible phenotype data have been uploaded to a tomato mutant database 'TOMATOMA' (http://tomatoma.nbrp.jp/), and these mutant seeds are available via this database. To provide important and valuable information for fruit research, we are also accumulating metabolic profiles of the tomato mutants, including amino acid composition, carotenoid contents and Brix values. In addition to the Micro-Tom mutants, we started to provide T-DNA tag lines of Micro-Tom recently. On the other hand, as DNA resources, the sequence information of Micro-Tom full-length cDNA and EST is available from database 'KaFTom (http://www.pgb.kazusa.or.jp/kaftom/) and EST database 'MiBASE' (http://www.pgb.kazusa.or.jp/mibase/), respectively. A reference genome sequence of Micro-Tom will be available through 'TOMATOMICS' (http://bioinf.mind.meiji.ac.jp/tomatomics/). These tomato resources will accelerate research and development of tomato and fleshy fruits.

P-TO-8

Fruit Ripening Regulation of α-Mannosidase expression by the MADS Box Transcription Factor RIPENING INHIBITOR and Ethylene

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α-Mannosidase (α-Man), a fruit ripening-specific N-glycan processing enzyme, is involved in ripening-associated fruit softening process. However, the regulation of fruit-ripening specific expression of *α-Man* is not well understood. We have identified and functionally characterized the promoter of tomato (*Solanum lycopersicum*) *α-Man* to provide molecular insights into its transcriptional regulation during fruit ripening. Fruit ripening-specific activation of the *α-Man* promoter was revealed by analysing promoter driven expression of *beta-glucuronidase* (*GUS*) reporter in transgenic tomato. We found that RIPENING INHIBITOR (RIN), a MADS box family transcription factor acts as positive transcriptional regulator of *α-Man* during fruit ripening. RIN directly bound to the *α-Man* promoter sequence and promoter activation/α-Man expression was compromised in *rin* mutant fruit. Deletion analysis revealed that a promoter fragment (567 bp upstream of translational start site) that contained three CArG boxes (binding sites for RIN) was sufficient to drive *GUS* expression in fruits. In addition, *α-Man* expression was down-regulated in fruits of *Nr* mutant which is impaired in ethylene perception and promoter activation/*α-Man* expression was induced in wild type following treatment with a precursor of ethylene biosynthesis, 1-*aminocyclopropane*-1-carboxylic acid (ACC). Although, *α-Man* expression was induced in *rin* mutant after ACC treatment, the transcript level was less as compared to ACC-treated wild type. Taken together, these results suggest RIN-mediated direct transcriptional regulation of *α-Man* during fruit ripening and ethylene may acts in RIN-dependent and -independent ways to regulate *α-Man* expression.

P-TO-9

Suppression of ADP-glucose pyrophosphorylase genes affects fruit skin thickness as well as fruit sugar and sugar phosphate contents in tomato (*solanum lycopersicum L.*)

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ADP-glucose pyrophosphorylase (AGPase) is a key regulatory enzyme in starch biosynthesis in plant. In tomato fruit, starch accmulattion at early developing stage is important for sugar content in red-ripe stage. We reported two genes encoding ADP-glucose pyrophosphorylase (AGPase), *AgpS1* and *AgpL1* are involved in the starch accumulation in fruit. However, the physiological function of starch and AGPase have not been well investigated in tomato to date. With this aim, in the present study, we generated RNAi transgenic tomato lines with suppressed expression of the *AgpS1* and *AgpL1* genes, and investigated metabolic alterations in developing fruits. Detailed metabolic characterization in a starch deficient line, *35S::AgpS1^{RNAi}* no. 67, revealed that soluble sugars and glucose-1-phosphate contents were respectively decreased by 19-27% and 19-22% in the transgenic compared to the wild-type at the red-ripe stage. Additionally, fruit malate content increased by about 30% in the RNAi lines compared to the wild-type fruit at immature-green and ripening stages, when the respiratory activity increases. Those results indicate i) that the contribution of starch to the fruit sugar content is about 30%, and ii) that glucan phosphorylase is involved in the starch degradation process, which occurs at early ripening in the fruit. Furthermore, the increase in malate, which was observed in the transgenic fruit suggests that there is a trade-off between starch and malate in developing fruits. Interestingly, the starch deficient lines exhibited reduced fruit skin thickness and hemicellulose content at red ripe stage. Those results indicate multiple roles of starch degradative product in tomato plant.

P-TO-10

Suppression of Tomato Prolyl 4 Hydroxylase 3 results in alterations on fruit development, ripening and abscission

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Proline hydroxylation is a major post-translation modification of hydroxyproline-rich glycoproteins (HRGPs) that is catalyzed by prolyl 4-hydroxylases (P4Hs). Their involvement in plant growth and development has been recently investigated in Arabidopsis, tobacco and carnation while little is known about their role in tomato. The tomato genome comprises 10 putative P4Hs with most of them being expressed during fruit development. Preliminary experiments to partially suppress their expression using Virus Induced Gene Silencing resulted in alterations on cell division and expansion of tomato leaves. Therefore, transgenic tomato plants expressing an RNAi construct were produced in order to suppress tomato P4H3 highly expressed during fruit development and ripening. Several independent lines down-regulating the target P4H were identified and nine of them were further characterized. The expression of the target P4H was completely suppressed during fruit ripening and the total hydroxyproline content in fruits was lower in most of the lines. All of the lines exhibited a reduction in fruit diameter while the number of viable seeds was significantly reduced. Moreover, a delay was observed in pedicel abscission which was associated with expression of key abscission progression genes. Collectively, these results indicate that the target P4H3 plays a significant role in tomato fruit development and abscission.

Parallel Session : Potato		
Genome-wide association studies using high-throughput SNP analysis in tetraploid potato	Sanjeev Kumar Sharma	P-PO-1

P-PO-1

Genome-wide association studies using high-throughput SNP analysis in tetraploid potato

Sanjeev Kumar Sharma[a], Micha Bayer[a], Karen McLean[a], Katrin McKenzie[b], Steve Daniels[c], Finlay Dale[a], Glenn Bryan[a]

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The cultivated potato is a highly heterozygous outbreeder, which exhibits tetrasomic inheritance. Due to the genetic intricacies posed by polyploidy potato genetics is often performed at the diploid level using bi-parental populations. Translating outcomes from diploid level studies to the commercially practised tetraploid level is not always straightforward, notably where allele dosage is important, such as the many economically important complex quality and physiological traits. The accurate dissection of traits at the tetraploid level across a wide range of germplasm is highly desirable. Genome-wide association studies (GWAS) hold great promise for studying the genetics of natural variation and agronomic traits. It also offers significant advantages, such as enhanced mapping resolution, increased numbers of segregating traits, and greater allelic diversity than traditional mapping using bi-parental crosses. However, a careful assessment of population structure in GWAS is required to avoid spurious associations arising from systematic variances in allele frequencies due to differences in sample ancestries. We have developed an association mapping panel of ~350 diverse autotetraploid potato cultivars and breeding lines and have exploited this resource for a large GWAS study based on single nucleotide polymorphism (SNP) markers. Various GWAS models have been examined. Insights from this study including an assessment of diversity, genomic complexity, population structure and a genome-wide survey of linkage disequilibrium will be presented.

Parallel Session - Pepper, Eggplant		
Assessment of Genetic Diversity in Pepper Germplasm using Genotyping-by-sequencing (GBS)	Jin-Kyung Kwon	P-PE 1
Genetic mapping of a novel locus controlling pungency of pepper (*Capsicum annuum*)	Byoung-Cheorl Kang	P-PE 2
Comparison Study of Resistance Gene Repertories in Capsicum Species using Resistance Gene Enrichment Sequencing (RenSeq)	Joung-Ho Lee	P-PE 3
Development of a visible reporter system for tracing virus-induced gene silencing as use of purple-colored pepper	Jihyun Kim	P-PE 4
Toward unveiling genetic determinant(s) of immature fruit color in pepper	Hee Ju Yoo	P-PE 5
Molecular Mechanisms Underlying Colour Development in Capsicum Fruit	Rebecca Nohl	P-PE 6

P-PE-1

Assessment of Genetic Diversity in Pepper Germplasm using Genotyping-by-sequencing (GBS)

Koeun Han[a], Heayoung Lee[a], Jin-Kyung Kwon[a], and Byoung-Cheorl Kang[a]

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Pepper (*Capsicum* spp.) germplasm shows diverse phenotypic variations including fruit size, color, pungency, and many other horticultural traits. Traditional markers including SSR, AFLP, and RFLP have been used to construct genetic maps using biparental populations. However to assess the genetic diversity of large number of germplasm, a robust and rapid marker development and genotyping approach is needed. We used six pepper accessions including *C. annuum*, *C. chinense*, *C. baccatum* and *C. frutescens* and performed genotyping-by-sequencing (GBS). To select the most appropriate condition, seven different selective primers with 2 bp selective nucleotides were used to make GBS libraries. One selective nucleotide showed the largest number of sequencing reads in all samples, and 11,026 to 47,957 high-quality SNPs were called in six accessions. When *C. annuum* 'CM334' genome sequence was used as a reference, *C. annuum* showed the smallest number of SNPs, while *C. baccatum* which was known to be a different *Capsicum* clade showed the largest number of SNPs. Pepper core collection chosen to represent the genetic diversity of whole germplasm will be genotyped by high-density SNPs developed from GBS. We will perform genome-wide association study (GWAS) using genetic and phenotypic variation to identify the functional genetic loci controlling horticultural traits.

P-PE-2

Genetic mapping of a novel locus controlling pungency of pepper (*Capsicum annuum*)

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Capsaicinoid is a unique compound of hot peppers (*Capsicum* spp.) and it gives burning sensation called pungency. Due to the pungency, hot pepper is consumed worldwide as a fresh vegetable, food ingredients, and also pharmaceuticals by the pharmacological effects including anti-cancer and anti-obesity. The *Pun1* gene have been identified to control the presence/absence of pungency in cultivated peppers including *C. annuum* and *C. chinense*, while the *Pun2* locus controls the pungency in *C. chacoense*. In this research we identify a novel locus controlling pungency by using a high-density genetic map. A total of 92 recombinant inbred lines (RILs) derived from a cross between non-pungent line *C. annuum* 'YCM334' and pungent line *C. annuum* 'Tean' and parental lines were sequenced by genotyping-by-sequencing (GBS) method and 1,609 high-quality SNPs were detected. Total genetic distances covered by the SNPs were 2,863 cM and the average distance between SNPs were 1.8 cM. Presence/absence of pungency in RILs was evaluated using Gibb's reagent, which shows the color reaction with the capsaicinoid. A 1:1 segregation ratio between pungent and non-pungent RILs showed the presence of a single locus controlling pungency. The *Pun1* gene was expressed normally in both parental lines. Therefore we mapped the locus using high-density genetic map and the phenotyping data. The novel locus was mapped on chromosome 7 and it is 5.8 cM apart from the closest SNP marker. We named this locus as *Pun3*, and further research will be done to fine-map the region.

P-PE-3

Comparison Study of Resistance Gene Repertories in *Capsicum* Species using Resistance Gene Enrichment Sequencing (RenSeq)

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Nucleotides binding-site leucine-rich repeat (NB-LRR) genes are important in plants because most dominant resistances are controlled by those genes. Resistance gene enrichment sequencing (RenSeq) is a promising method to find NB-LRR gene complements in plant genome. To design an Agilent SureSelect enrichment bait library, 755 predicted NB-LRR gene complements from the gene annotation file were used. Before genome-wide comparison study, re-annotation of NB-LRR gene complements in pepper reference genome (*Capsicum annuum* 'CM334') identified ~300 more NB-LRR gene complements than gene annotation file based data. After re-annotation step finished, RenSeq data from six common *Capsicum* species (*C. annuum*, *C.chinense*, *C.baccatum*, *C.frutescens*, *C.pubescens*, *C.chacoense*) enabled the comparison of NB-LRR gene complements. It seemed to share most NB-LRR gene complements in six *Capsicum* species, but there was a few exception that only existed in some *Capsicum* species. This data indicated that evolutionary process has occurred in different times on some NB-LRR genes. We are comparing the sequences of six *Capsicum* species in detail and studying diversity in the domain architectures of these NB-LRR genes. Finally, these data offer further insights into evolutionary work of NB-LRR domains among not only in a Capsicum species but also in the Solanaceae family.

P-PE-4

Development of a visible reporter system for tracing virus-induced gene silencing as use of purple-colored pepper

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Virus-induced gene silencing (VIGS) has become a choice of post-genomic technology for plant species, which are recalcitrant to Agrobacterium-mediated transformation such as pepper. Although VIGS in plants has being widely employed as a powerful tool of functional genomics, scattering phenotypic effects by uneven gene silencing has been raised to overcome especially in fruit tissue. We improved VIGS system based on tobacco rattle virus (TRV) containing *AN2* myb transcription factor, which is the genetic determinant of purple colored- or anthocyanin rich-pepper. The silencing of endogenous *AN2* in the purple-colored pepper by the modified TRV infection was lead to lack of purple color in leaves, flowers and fruits. Infection with TRV containing a tandem construct of *AN2* and phytoene desaturase (*PDS*) resulted in a typical photobleaching in leaves without purple pigments whereas the silencing of *PDS* only showed photobleached and purple-colored leaves. The dual silencing of endogenous *AN2* and *capsaicin synthase* in fruits resulted in decreased level of capsaicin and dihydrocapsaicin coupled with lack of purple pigments in fruits. Taken together, these results indicate that VIGS with tandem constructs harboring *AN2* as a visible reporter can accelerate a functional genomics in the study of metabolic and fruit biology in pepper.

P-PE-5

Toward unveiling genetic determinant(s) of immature fruit color in pepper

Hee Ju Yoo[a], Gyu Myung Lee[a], Jihyun Kim[b], Doil Choi[b] Je Min Lee[a]

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Pepper fruit quality is a major trait to consumers, and attributes to appearance, taste, flavor, metabolic composition and nutritional value. Among the quality traits, fruit color has a primary importance because the pigments coferring color are associated with nutrition, health and flavor. Unlike mature fruit color, genetic basis of immature fruit color is largely unknown, which hesitates its utilization for pepper improvement. We mined candidate gene(s) impacting immature fruit color using comparative genomics approach between tomato and pepper. Candidate gene function impacting immature fruit color was confirmed by virus induced gene silencing in fruits. We are currently validating its genetic potential in multiple F2 population and germplasms showing large variation of immature fruit color. We will present detailed results and its utilization in pepper molecular breeding.

P-PE-6

Molecular Mechanisms Underlying Colour Development in Capsicum Fruit

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Colour is an important consumer trait conferring value on fruit crops, including the sweet bell pepper (*Capsicum annuum*). Carotenoids, the class of isoprenoids responsible for the distinctive orange, yellow and red colours of the fruit, also have important roles to play in health and nutrition. Therefore, the mechanisms regulating their formation in pepper should be thoroughly understood. Mature fruit colour in *Capsicum* is controlled by three loci: *c1/C1*, *c2/C2*, and *y/Y* (Hurtardez-Hurndando and Smith, 1985). The *C2* locus has been associated with the fruit specific enzyme catalysing the first step of carotenoid biosynthesis, phytoene synthase (PSY-1) (Huh et al., 2001). In the present work, a discovery panel of sixteen Capscium annuum accessions ranging in colour from red to white was used to further investigate the role of PSY-1 in determining pepper mature fruit colour. Reverse-phased chromatography and quantitative real-time PCR were used to investigate the molecular mechanisms governing colour in this panel of lines. The structure of the plastids in which carotenoids accumulate and the specialisations of their sub-compartments were investigated with sub-chromoplast fractionation, electron microscopy, and enzyme activity assays. Results of these experiments reveal some striking differences in regulation of early steps in the pathway between pepper and its close relative tomato.

Parallel Session - Specialized Metabolism		
Characterization of aminotranferase involved in steroidal glycoalkaloids biosynthesis in *Solanum* plants	Masaru Nakayasu	P-SM-1
Biochemical and Ultrastructural Studies of a Novel Tomato Mutant *pale yellow petal* 2 Revealed that Xanthophyll Accumulation are Important for Normal Chromoplast Differentiation.	Satomi Takezawa	P-SM-2

P-SM-1

Characterization of aminotranferase involved in steroidal glycoalkaloids biosynthesis in *Solanum* plants

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Potato (*Solanum tuberosum*) and tomato (*S. lycopersicum*) are known to contain steroidal glycoalkaloids (SGAs), which are natural toxins and composed of C27 steroids with a nitrogen-containing heterocycle and an oligosaccharide at the hydroxy group of the C-3 position. Potato accumulates α-solanine and α-chaconine and tomato contains α-tomatine. These SGAs are thought to be biosynthesized from cholesterol by the oxidations at positions C-16, 22, and 26, the transamination at C-26, the cyclization of E-, and F-rings, and the glycosylation at the C-3 hydroxy group. However, little is known about enzymes and genes for SGA biosynthesis. Previously, we identified two cytochrome P450 genes (*SGA1*, *SGA2*) and dioxygenase gene (*16DOX*), which are involved in the oxidation steps of cholesterol at the C-26, C-22 and C-16 positions, respectively. In this study, to explore the other genes involved in SGA biosynthesis, we selected an aminotransferase (*SGA4*). *SGA4* cDNA encodes GABA-aminotransferase 2 (GABA-T2). Potato *SGA4* gene is highly expressed in tuber sprouts and tomato *SGA4* gene is highly expressed in flowers, and thus the *SGA4* genes are coexpressed with the genes involved in SGA biosynthesis. The *SGA4*-knockdown transgenic plants exhibited the decrease of the endogenous SGA contents. Biochemical characterization of the recombinant SGA4 protein will be presented. (201 words)

P-SM-2

Biochemical and Ultrastructural Studies of a Novel Tomato Mutant *pale yellow petal 2* Revealed that Xanthophyll Accumulation are Important for Normal Chromoplast Differentiation.

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Xanthophylls are a group of carotenoid that contains hydroxyl residues in their molecular structures and act as main pigments in reproductive organs of tomato (*Solanam lycopersicum*) petal. It is revealed that xanthophylls accumulate in the petal in esterified form, but the molecular mechanism of esterification process has been poorly understood. We isolated three alleles of novel tomato mutant *pale yellow petal 2* (*pyp2*) that show reduced yellow pigmentation both in petal and anthers. HPLC analysis of carotenoid extract from WT and *pyp2* mutant petal with saponification revealed that they accumulate free xanthophylls, *trans*-, *cis*-neoxanthin and violaxanthin. Without saponification, xanthophyll esters were detected in both WT and *pyp2* mutants in addition to free xanthophylls. However, the amounts of free xanthophylls and xanthophyll esters were lower in *pyp2* mutant than WT. Next, we compared the mRNA expression levels of carotenogenic genes in the petal of WT and *pyp2* mutants by using qRT-PCR analysis. The expression profiles of these carotenogenic genes were not significantly different between WT and *pyp2* mutants. We observed ultrastructural changes during plastid to chromoplast in WT and *pyp2* mutants by using TEM and appeared that differentiation of plastid to chromoplast was prevented; plastoglobule formation in chromoplast was barely observed in *pyp2* mutant. These results show that reduced petal pigmentation of *pyp2* mutants were due to the reduction of total carotenoid amounts. In addition, disruption of normal chromoplast growth might be associated with lower carotenoid levels in *pyp2* mutants.

Parallel Session - Tobacco		
Reducing TSNAs in Burley Tobaccos through Alteration of the N-Assimilation Pathway	Bovet L	P-TB-1
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P-TB-1

Reducing TSNAs in Burley Tobaccos through Alteration of the N-Assimilation Pathway

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Burley tobaccos require a much higher level of N-fertilization than other tobacco types in order to achieve acceptable yields. As a consequence, Burley plants accumulate higher levels of free nitrate in their leaves than other tobacco types. In the literature high levels of nitrate have been correlated with high nitrosation of alkaloids leading to the formation of tobacco-specific nitrosamines (TSNA) which are reported for their carcinogenic activity.
In order to investigate the relationship between nitrate levels and nitrosation and to reduce TSNAs in tobacco leaf and smoke, a strategy was developed to deplete the pool of nitrate accumulated in Burley leaf by altering the N-assimilation pathway. Nitrate reductase (NR) is the first key controlled enzyme of the N-assimilation pathway reducing nitrate to nitrite. A deregulated form of S523DNR was overexpressed in Burley tobacco under the control of a constitutive promoter to increase nitrate accumulation into amino acids and thereby reducing the free nitrate pool accumulated in leaf, expecting thus a decrease in nitrosating agents responsible for TSNA formation.

P-TB-2

Missense or nonsense mutations in either *HMA4.1* or *HMA4.2* do not significantly reduce cadmium content in the leaves of tobacco plants grown under field conditions

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The heavy metal cadmium (Cd) is classified as a human carcinogen interfering with the roles of essential metals. The reduction of Cd is therefore desirable in crop plants in order to limit human exposure. It has been shown that one nonsense or missense mutation in either of the two tobacco (*Nicotiana tabacum*) heavy metal ATPase transporter genes *HMA4.1* or *HMA4.2* leads to 50% Cd reduction in leaves, when tobacco plants are grown on agar medium or in hydroponics containing very high Cd concentrations (Hermand et al., 2014, *Metallomics* 6(8): 1427-1440; WO2012/041913). The aim of the present study is to determine the applicability of these findings to commercial tobacco production under field conditions, where Cd and zinc concentrations are much lower compared to artificial growing conditions (agar media, hydroponics). Plants harbouring a homozygous missense or nonsense mutation in one of the *NtHMA4* genes were tested in field trials. No significant reduction was observed in any of the tested mutant lines containing a homozygous missense or nonsense mutation in one of the *NtHMA4* genes. Our conclusion is that the artificial high Cd conditions used by Hermand et al. (2014) do not reflect the natural conditions that are present in an open field and consequently, a deleterious mutation in one of the *NtHMA4* genes does not significantly reduce Cd levels in plants grown under field conditions.

P-TB-3

Properties of *Nicotiana glauca* as a biorefining feedstock

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Increasing atmospheric CO_2 levels, temperature and raising sea levels has been pressurising societies to increase sustainability in the environment. *Nicotiana glauca* is a potential feedstock for biorefining, it has the advantage of relatively high quantities of long chain hydrocarbons (C_{29}-C_{33}) and a simple plant composition of fatty acids which makes this plant material a potential source for biofuel particularly aviation blended fuel. This species will grow on margin land and will not compete with food production, has a wide range of valuable secondary metabolites and is amenable to genetic manipulation. In this study analyses of the non–polar fraction from *N. glauca* over ten stages of leaf development. The study also looks into summer and winter cultivated crops and metabolite changes at key stages of leaf development. The analysis demonstrate a total 20% of hydrocarbon content in non–polar metabolite composition, of which 94% was hentriacontane. Further analyses showed high levels of phytol, C16:0 palmitic acid, C18:2 cis 9,12 linoleic acid and relatively high carotenoid and sterol content. Meanwhile, comparing summer and winter harvests, a decrease in fatty acids, phytol and phytoene was observed in winter crop. On the contrary, an increase of sterols and most carotenoids was recorded in winter harvest. Overall, no significant changes were observed between summer and winter crops in hydrobcarbon quantities. It is concluded that *glauca* does not require a specific stage or season of harvest for biorefining processes and in addition to biofuels other metabolites and commodities are present that can act as valuable side streams.

P-TB-4

Genomic changes generated in natural and synthetic *Nicotiana* allotetraploids : what do transposable elements tell us ?

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Allopolyploids originate from hybridization between divergent genomes associated with chromosome set doubling. Their genomes may undergo a wide range of structural, epigenetic and functional changes. As transposable elements (TEs) are major components of plant genomes, they may play a key role in the genetic and functional modifications produced by the allopolyploidy process.
We assessed the extent of genomic changes associated with TEs in three recent allopolyploid species of the *Nicotiana* genus : *N. rustica*, *N. arentsii* and *N. tabacum* (tobacco), for which it is possible to create *de novo* synthetic hybrids from the extant accessions of parental species. To unravel the extent of TE-associated structural changes, we performed comparative analysis of SSAP (Sequence-Specific Amplification Polymorphism) profiles obtained for six different endogenous TE populations in both natural and synthetic accessions as well as their diploid progenitors. In natural young *Nicotiana* allopolyploids, each TE family displays a specific evolutionary trajectory. The loss of parental bands is the main event and levels of new bands roughly reflect what is known about the dynamics of each TE. TE divergence between progenitors is also strongly correlated with TE-associated restructuring levels, in agreement with the genome shock model. In *Nicotiana* synthetic hybrids, we observed mainly additive profile as expected but also some TE-related genomic restructuring in F1 hybrids, indicative of TE-associated genome reorganization at early hybridization steps.

P-TB-5

Genetic and functional analysis of resistance to *Potato virus Y* in tobacco

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Potato virus Y (PVY, *Potyvirus*) is distributed worldwide and is a major pathogen of tobacco[1]. To date, the main source of resistance against PVY is the *va* gene. This gene is within a deletion of almost 1Mb originating from the VAM tobacco genotype. Recently, Julio et al.,[2] showed that a deletion of the *S10760* gene encoding a copy of the eukaryotic initiation factor 4E is responsible for *va*-mediated resistance to PVY. We compared the durability of the resistance conferred by various types of mutations affecting the *S10760* gene such as nonsense mutations, natural frameshift mutants or a smaller genomic deletion than the one found in *va* plants. The results obtained reveal significant differences in resistance durability between tobacco genotypes carrying the different types of mutations, the *va* form being the most durable. However, the resistance-breaking PVY isolates emerging in the different tobacco genotypes appear to accumulate similar mutations, suggesting that resistance stability but not resistance-breaking mechanisms is affected by the nature of the mutation affecting the *S10760* gene.
Moreover, screening of a large collection of tobacco accessions allowed the identification of 11 resistant genotypes expressing a *VA* susceptibility allele, suggesting that the observed resistance does not rely on *va*. After challenging those 11 accessions, we observed a phenotype of tolerance rather than resistance to PVY (lack of necrotic symptoms but detection of viral accumulation). The genetic characterization of those non-*va* tolerant accessions is underway, as a potential new source of resistance to PVY beside *va*, in future tobacco breeding programs.

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The phytobiomic basis of bacterial wilt resistance in tomato

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The three elements of the disease triangle for an epidemic constitute a susceptible plant host, a virulent pathogen, and a favorable environment such as moisture and temperature. Among the environmental factors, biotic factors, e.g., commensal microbiota that inhabits the plant, may significantly contribute to disease development. To test this possibility, we initiated a whole metagenomic analysis of microbial communities in the rhizosphere of two tomato cultivars, Hawaii 7996 and Moneymaker, which are either resistant or susceptible to a bacterial wilt caused by Ralstonia solanacearum. Taxonomic comparison of the recruited 16S rDNA sequences demonstrated that relative abundance of the class Flavobacteriia is higher in the rhizosphere of Hawaii 7996 than that of Moneymaker. On the other hand, relative abundance of Bacilli and Betaproteobacteria were higher in the rhizosphere of Moneymaker. These tendencies cohered with the results from the reference genome-guided analysis. To compare the gene contents, de novo assembly and gene prediction followed by COG and Subsystem assignments were conducted. When the scaffolds were sorted according to the bacterial phyla, scaffolds assigned to Bacteroidetes had higher fold-coverage in Hawaii 7996 than Moneymaker. Paired-end reads of the scaffolds of Bacteroidetes in Hawaii 7996 were extracted and re-assembled, to reveal the genome of an unclassified Flavobacteraceae bacterium. The genome had a high proportion of genes involved in carbohydrate metabolism or transport. These results suggest that a unique microbial community forms in the tomato rhizosphere in a cultivar specific manner and the microbiome function plays a pivotal role in suppressing the soil-borne disease.

P-V-9

Tomato AUXIN RESPONSE FACTOR 5, a critical gene that regulates flower formation and development in tomato

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Flower formation and development significantly affect the crop's yield in agricultural plants. Auxin as a basic plant hormone is pivotal to regulate these processes. It functions strictly through a hierarchical signal pathway, including auxin synthesis, polar auxin transport, auxin perception and auxin response. The mutation of AUXIN RESPONSE FACTOR5 (ARF5) leads to the pin-like structure in Arabidopsis inflorescence, suggesting ARF5 is a master transcription factor in controlling flower formation in auxin signal pathway. However, little is known for its regulation network in downstream. Here, we identified a homologuous gene, Sl-ARF5. Using a single cell approach, we found SlARF5 is located both in nucleus and cytoplasm. Transcriptomic data and quantative qPCR showed that Sl-ARF5 is highly expressed in floral meristem and flowers, respectively. Using reverse genetic approach, we showed that down-regulation of Sl-ARF5 in tomato leads to strong deficiency in flowers morphology where petals were lost, the number of sepal and stamens were significantly reduced, and split stamen or carpel were formed. These data indicates that Sl-ARF5 is critical for flower development in tomato. To find its direct target genes, ChIP-seq is planned to be performed in transgenic tomato flowers overexpressing a GFP-tagged Sl-ARF5 protein.

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